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# HEMAGGLUTINATION STUDIES WITH SCHISTOSOME ANTIGENS

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The tannic acid hemagglutination test (Boyden, 1951), utilizing saline extracts of schistosome cercariae as antigen, proved to be a sensitive serological test for the detection of antibodies in the sera of rabbits injected with stages from the life cycle of *Schistosoma mansoni* and *Schistosomatium douthitti,* (Kagan, 1955a). Additional studies were made with other schistosome antigens tested against sera from rabbits injected with stages of *S. mansoni* and against sera from naturally infected humans. A preliminary abstract of these results has been published (Kagan and Oliver-González, 1955).

In the evaluation of serological tests for schistosomiasis it should be noted that various anigens and procedures appear able to detect different antibodies in the serum of infected hosts. In the precipitin test, stage-specific antibodies may be detected with homologous antigens in antisera of rabbits injected with cercariae, adults, or eggs of *S. mansoni.* Cercarial agglutinin and cercarial precipitin titers are higher in acute human cases compared to chronic infections (Oliver-González, et al, 1955a). In the circumoval precipitin test (Oliver-González, 1954) species-specific antibodies were detected with homologous eggs in human antiserum (Oliver-González, 1955). During active infection with *S. mansoni,* skin tests made with egg antigen were negative, whereas skin tests with cercarial antigen were positive (Oliver-González, et al, 1954). Several months after chemotherapeutic cure, the skin test with egg antigen became positive and the circumoval precipitin test became negative (Oliver-González, et al, 1955b). These findings are of interest in the serology of schistosomiasis and in the sero-evaluation of chemotherapeutic cure. In the present study, the hemagglutination technique was evaluated as a serological method for the detection of stage-specific antibodies, as a technique for determining chemotherapeutic cure, and as a method for the diagnosis of infection.

## MATERIALS AND METHODS

Seven rabbits were artificially immunized with lyophilized eggs, cercariae or adult antigens of *S. mansoni* suspended in Freund's adjuvant (Bayol F plus Arlacel A and BCG, Cohn, 1953) and the antisera were used in the hemagglutination test. Rabbits 176 and 198 were injected 6 times with 2 mg of dry egg antigen per inoculum, 183, 192 and 199 with similar amounts of cercarial antigen, and 194 and 182 with adult anitgens. Samples of serum were tested from 5 children with active infections of *S. mansoni* detected by finding eggs in the stools. Serum was also collected from 10 adults who were passing eggs in their stools. These adults were treated

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and cured of their infections and 1 year later additional serum samples were collected. Sera were therefore obtained during active infection and 1 year after cure.

The following lyophilized anigens in phosphate-buffer-saline (0.85% NaCl, pH 7.2) in dilutions of 1:5000 (w/v) were employed as test antigens: Cercariae, adults, eggs of *S. mansoni*, eggs of *S. haematobium*, and eggs of *S. douthitti* (from spleens of mice). Metabolic-product antigens were also prepared by incubating 100,000 cercariae and 100 adults of *S. mansoni* in 10 ml of saline for periods of 24 hours and using the fluid as antigen. Eggs of *S. mansoni* and *S. haematobium* were isolated from liver tissue, cercariae from infected snails, and adults from infected mice or hamsters.

Boyden's tannic acid hemagglutination technique was used (Boyden, 1951). Sheep cells in Alsever's solution were washed 3 times in phosphate-buffered saline (pH 7.2) and were incubated at 37° C for 10 minutes with a 1:20,000 or 1:40,000 tannic acid solution. After centrifugation the packed, tanned cells were exposed to 5 volumes of a 1:5000 antigen (0.001 mg of antigen per 5 ml of saline buffered at pH 6.4 or 7.2) for 15 minutes at room temperature. After centrifugation, antigen-coated cells were adjusted to a 2% suspension in 1:250 normal rabbit serum. Antisera were tested in serial dilution with 1:100 normal rabbit serum as the diluent. One drop of antigen-treated cells was added with an Ives pipette to each tube (10×70 mm) containing 0.5 ml of antiserum. The tests were read at room temperature after 2 hours and after being placed in a cold room (4° C) over night. Once each day 2 control tests were made with tanned red cells and tanned antigen-coated cells against normal rabbit or human serum. End points of the hemagglutination test were at times accentuated by replacing the serum on the second day with saline without discarding the red cells in the tube, shaking the cells and reading the agglutination titer in 2 hours. The titers reported in the tables are averages of several tests.

Sheep cells were used in all tests for which data are reported. Some experiments were made with type O human cells obtained from the blood bank; these cells did not appear to differ in their reactivity in the test from sheep cells.

Sera were also tested for CHR antibodies (Cercarienhüllen reaction of Vogel and Minning, 1949) by placing living cercariae in a drop of undiluted serum in a depression slide and observing after 12–24 hours of incubation for the presence or absence of a membrane around the body and tails of the cercariae (Kagan, 1955b).

Ring tests were made by carefully overlaying full strength serum with antigen in small microprecipitation tubes and observing the formation of a precipitate at the interphase after 30 minutes at room temperature.

## EXPERIMENTAL RESULTS

The highest hemagglutination titers were obtained with cercarial antigen when tested against rabbit antiserum. No specificity was evidenced with this antigen since cross-reactions were observed with anti-adult and anti-egg rabbit serum in both hemagglutination and ring tests. In the precipitin ring test, positive tests were obtained very quickly when antigen was tested against the homologous antiserum. No reaction was obtained with adult worm antigen against anti-egg serum in the precipitin test, but high titers were observed in the hemagglutination test. Egg antigens of 3 schistosome species showed no specificity in the hemagglutination test. Adult-metabolic antigen cross-reacted with rabbit cercarial antiserum but was not tested with egg antiserum. The CHR test was positive with all rabbit antisera tested (Table I).

Sera from 5 children with heavy infections of schistosomes were tested with cercarial, adult, and egg antigens of *S. mansoni* by means of the hemagglutination test. In 3 of 5 sera, the egg antigen showed the highest titers. Two sera were negative with the CHR test (Table II).

Sera from the 10 adult patients were tested by hemagglutination with egg antigens of *S. mansoni* and *S. haematobium*, adult and cercarial metabolic secretions antigens and a cercarial antigen of *S. mansoni*. Serum obtained during active infection and 1 year after cure were positive by hemagglutination with cercarial antigen and cercarial metabolic secretions antigen. Titers in all instances were slightly higher with

serum obtained before treatment. Four sera collected during active chronic infection (before treatment and cure) were positive by hemagglutination with egg and adult-metabolic secretions antigens (Table III).

TABLE I.—*Hemagglutination titers, precipitin ring tests and CHR tests with antiserum from rabbits injected with antigens from* Schistosoma mansoni.

| Rabbit | Antigen Injected | CHR | Precipitin Ring Test | | Hemagglutination Titers | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cercarial Antigen | Adult Antigen | *S. mansoni* Adult | *S. mansoni* Cercariae | *S. mansoni* Egg | *S. haemat.* Egg | *S. doun'h.* Egg | Adult Met. Prod. |
| 176 | Eggs | + | – | – | 1 : 640 | 1 : 320 | 1 : 80 | 1 : 160 | 1 : 40 | |
| 198 | Eggs | ++ | + | – | 1 : 80 | 1 : 80 | 1 : 40 | 1 : 160 | 1 : 320 | |
| 183 | Cercariae | ++ | ++ | – | 1 : 160 | 1 : 640 | 1 : 80 | | 1 : 160 | 1 : 40 |
| 192 | Cercariae | ++ | + | + | 1 : 80 | 1 : 1280 | | | | |
| 199 | Cercariae | + | – | – | 1 : 40 | 1 : 320 | | | | 1 : 160 |
| 194 | Adults | ++ | + | + | 1 : 160 | 1 : 1280 | 0 | | | |
| 182 | Adults | | + | + | 1 : 160 | 1 : 1280 | 1 : 80 | 1 : 160 | 1 : 320 | 1 : 40 |

TABLE II.—*Hemagglutination titers and CHR tests with sera of children infected with* Schistosoma mansoni.

| Serum | Cercariae Antigen | Adult Antigen | Egg Antigen | CHR |
|---|---|---|---|---|
| 1029 | 1 : 80 | 1 : 320 | 1 : 640 | ± |
| 1031 | 1 : 640 | 1 : 640 | 1 : 320 | + |
| 1032 | 1 : 80 | 1 : 20 | 1 : 640 | 2 + |
| 1033 | 1 : 1280 | 1 : 80 | 0 | – |
| 1035 | 1 : 640 | 1 : 160 | 1 : 1280 | – |

TABLE III.—*Hemagglutination titers of human sera collected during infection and one year after treatment and cure.*

| Serum | Antigens Used in Hemagglutination Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cercarial | | Cercarial Secretions | | Adult Secretion | | Egg *S. mansoni* | | Egg *S. haematobium* | |
| | Infected | Cured | Infected | Cured | Infected | Cured | Infected | Cured | Infected | Cured |
| 1 | 1 : 80 | 1 : 40 | | | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 : 80 | 1 : 40 | | | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 1 : 40 | 1 : 20 | 1 : 20 | 1 : 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 1 : 160 | 1 : 80 | 1 : 160 | 1 : 80 | 1 : 80 | 0 | 1 : 160 | 0 | 1 : 160 | 0 |
| 5 | | | 1 : 20 | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | | | 1 : 80 | 1 : 40 | 1 : 20 | 0 | 0 | 0 | 1 : 40 | 0 |
| 7 | | | 1 : 40 | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | | | 1 : 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | | | 1 : 40 | 0 | 1 : 80 | 0 | 1 : 80 | 0 | 0 | 0 |
| 10 | | | 1 : 320 | 1 : 40 | 0 | 0 | 1 : 20 | 0 | 0 | 0 |

## DISCUSSION

The sera employed in the hemagglutination test were the same sera prepared for studies of precipitin antibodies and their specificity by Oliver-González et al, (1955a). In some of their experiments the precipitin ring test titers were higher and more specific than the hemagglutination titers we found. Schistosome antigens in the hemagglutination test showed no specificity with stage-specific rabbit antisera. For diagnosis of acute infections in children, the egg antigen was more effective than the cercarial and adult antigens in the hemagglutination test. Egg antigens and adult metabolic-secretion antigens showed promise in the evaluation of chemotherapeutic cure with the hemagglutination test, but need more careful study since only 4 of 10 sera gave positive tests with these antigens.

The hemagglutination test is a sensitive test for the diagnosis of schistosome infections. With the proper antigens it may prove to be a valuable serological method for the evaluation of chemotherapeutic cure. Utilizing saline extracts of various stages as antigens, the hemagglutination test showed no specificity with stage-specific rabbit antisera. The significance of the titer in the hemagglutination test has not been statistically evaluated. It has been our experience that 2–3-tube differences in titer may be expected from day to day with some antigens.

## SUMMARY

Antisera prepared in rabbits against whole eggs and extracts of cercariae and adults of *Schistosoma mansoni* were tested with homologous and heterologous antigens by means of the hemagglutination test. Cross-reactions with egg antigens of 3 schistosome species, cercarial, and adult antigens of *S. mansoni* were obtained with these sera. With sera of children infected with *S. mansoni*, egg antigen of *S. mansoni* gave higher titers than cercarial or adult antigens. With sera of 10 adult patients obtained during chronic infection and 1 year after cure, egg antigens and an antigen from adult metabolic products were positive by hemagglutination with 4 sera taken during infection, but not after cure. With the development of more potent metabolic antigens, the hemagglutination test may prove an effective serological test in the valuation of chemotherapeutic cure.

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# STUDIES ON *CLONORCHIS SINENSIS*. III. THE HOST–PARASITE RELATIONS IN THE RABBIT AND OBSERVATIONS ON THE RELATIVE SUSCEPTIBILITY OF CERTAIN LABORATORY HOSTS*

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The Chinese liver fluke, *Clonorchis sinensis,* was first described by McConnel in 1875. In 1910, Kobayashi discovered the second intermediate host to be certain species of cyprinoid fish, and in 1918 Muto found the first intermediate host to be aquatic operculate snails. It has become established that the adult flukes live in the biliary passages of many mammalian hosts, including man. Cats, dogs and guinea pigs have been used for experimental infections, but the rabbit has been relatively untried as a laboratory host.

Investigators have generally infected animals with unknown numbers of metacercariae. Faust and Khaw (1927) reported ". . . only a small percentage of the originally viable cysts, possibly only 5% of the viable larvae taken into the stomach, actually reach the common duct." Hsü and Wang (1938) fed known numbers (from 3 to 250) to cats and reported 60% developed to maturity.

All known intermediate snail hosts of Clonorchis belong to the family Hydrobiidae (Amnicolidae). One of these, *Bulimus tentaculatus,* belonging to the same genus as a natural Far Eastern host (*B. fuchsianus*), has been found recently in the Great Lakes region and near Washington, D. C. That this snail perhaps may serve as an intermediate host has been noted by Chandler (1955). In addition, there are several other members of this snail family which perhaps may serve as intermediate hosts.

Contacts brought about by recent world events and the possibility of introducing this parasite to the U.S.A. have stimulated interest in the present problem, which was to reexamine earlier observations of host-parasite relationships, and to investigate some aspects on which information was lacking or inadequate.

## MATERIALS AND METHODS

Specimens of the fish *Pseudorasbora parva* which serve as second intermediate hosts for *Clonorchis* were collected in Okayama, Japan, and sent to the 406th Medical General Laboratory, Zama, Japan, where the flesh was digested by the technic of Komiya and Murase (1951). The metacercariae were iced and sent by air to Washington, D. C., for use in this and other work (Wykoff and Lepeš, 1957; Wykoff, to be published). Under the low power of a stereoscopic microscope, the *C. sinensis* metacercariae were sorted from the several other species (Komiya and Tajimi, 1941), drawn into medicine droppers with attenuated tips, counted and discharged into the throat of the experimental animal.

In order to count the adult worms, the liver of the sacrificed animal was first cut into thin strips and examined macroscopically. When all visible worms had been removed, small pieces of the liver were pressed between 2 plates of clear plastic. By using a small amount of tissue and holding the plates up to a light, the worms in the distal biliary passages could be seen. With

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infections of short duration, it was necessary to place the plates under a stereoscopic microscope to detect the small worms. This procedure appeared to give more accurate determinations than Khaw's (1930) method of squeezing the adults into saline for counting.

Four species of snails were obtained for infection trials. *Bulimus tentaculatus* were collected near Washington National Airport; *Gillia altilis* from Great Falls, Virginia; *Fluminicola coloradoense* from Salt Lake City, Utah; and *F. fusca* from Hanford, Washington. They and their progeny were placed in deep dishes containing large numbers of viable eggs washed from the bile of sacrificed cats. The dishes were covered with gauze to prevent the escape of the snails, and immersed in an aquarium for 24 hours. The gauze was then removed, permitting them to wander about the tank. Weekly, 1 to 4 months later, the snails were crushed for examination, excepting *F. coloradoense* which were examined when they died, generally 3 to 10 days after arrival and exposure. Hsü and Chow (1939) exposed snails in this manner and found rediae and cercariae 107 days later.

## RESULTS

*Metacercarial size and viability.* Studies by Binford (1934) indicated that the metacercariae would remain viable in fish sent from the Orient to Hawaii for human consumption, but the present work has, for the first time, required the deliberate shipment of the metacercariae freed from fish flesh, and observations were necessary to determine the length of time they would remain viable. An average of 16 days elapsed between the death of the second intermediate host and the arrival of the metacercariae in Washington, D. C. Viability of each shipment was determined by microscopic examination for larval motility and by animal infections. It was found that from the time of arrival until 40 days after death of the fish host there was no significant decrease in viability, and apparently 50% were viable after 60 days when kept at 3–6° C.

Faust and Khaw (1927) reported that the metacercariae of *Clonorchis* measured 0.26–0.28 × 0.20–0.22 mm; Hsü and Khaw (1936) gave 0.121–0.150 × 0.085–0.150 mm, and the largest measurements given by Kobayashi (1917) were 0.13–0.145 × 0.09–0.10 mm. The average size of 500 metacercariae used in the present study was 0.166 × 0.148 mm. These measurements and those of Hsü and Khaw were made with living material without coverslip pressure. It was not stated how the measurements were made in the other studies. If the measurements were made on compressed or fixed specimens the difference in observed sizes may have been due to these factors.

*Susceptibility of definitive hosts.* In determining the relative suceptibility of the definitive hosts, 10 rabbits, 12 guinea pigs and 11 white rats were given from 10 to 1000 metacercariae each (Table I). All of four guinea pigs given 1000 metacercariae died from the infection, on the 16th, 19th, 21st and 24th days, respectively. One of three rabbits given 1000 metacercariae died on the 43rd day; the other two survived over 90 days before being sacrificed. All rats survived the experimental infections.

When sacrificed, two rabbits given 10 metacercariae each yielded 2 and 3 adults; two others given 50 metacercariae yielded 5 and 8. Inocula of 100, 300 and 500 yielded, respectively, 15, 60 and 240 adults, while three given 1000 metacercariae each yielded 301, 315, and 454 adult worms. From two guinea pigs given 10 metacercariae each, 0 and 2 adults were recovered. From inocula of 100, 300, 500 and 500 metacercariae, 26, 153, 214 and 235 adults were obtained, respectively, while four animals receiving 1000 cysts yielded 188, 305, 310 and 331 adults at the time of death. Two rats given 50 metacercariae yielded 3 and 5 adults, whereas two given 100 yielded 11 and 12. With inocula of 300, 500 and 500 metacercariae, 35,

11 and 30 adults were recovered, respectively. Four rats given 1000 cysts each yielded 12, 36, 92 and 101 adults. It appears that rabbits and guinea pigs are about equally susceptible (35 and 32% of the metacercariae developing to maturity, respectively), while rats appear to be more refractory (6% development). Apparently an inoculum of 1000 metacercariae is lethal to guinea pigs.

*Host-parasite relations.* The prepatent period was determined in 12 rabbits and 10 guinea pigs. Examinations for eggs were made with the Weller-Dammin (1946) acid-ether concentration technic until eggs were found in the feces. In 12 rabbits given 10 to 1000 metacercariae, the prepatent period ranged from 19 to 29 days, with a mean of 22 days. There appeared to be no correlation between the size of the inoculum and the prepatent period. For comparison, similar observations were made with guinea pigs. The range of prepatent period was 16 to 31 days with a mean of 20 days. Again, there appeared to be no correlation between size of inoculum and prepatent period.

TABLE I. *Percentage of orally administered* Clonorchis sinensis *metacercariae developing to maturity in rabbits, guinea pigs, and white rats.*

| Number of metacercariae given: | Number and percentage ( ) of metacercariae recovered as adults in | | |
|---|---|---|---|
| | Rabbits | Guinea pigs | White rats |
| 10 | 2 (20) | 0 ( 0) | .. .. |
| 10 | 3 (30) | 2 (20) | .. .. |
| 50 | 5 (10) | 6 (12) | 3 ( 6) |
| 50 | 8 (16) | 10 (20) | 5 (10) |
| 100 | 15 (15) | 26 (26) | 11 (11) |
| 100 | .. .. | .. .. | 12 (12) |
| 300 | 60 (20) | 153 (51) | 35 (12) |
| 500 | 240 (48) | 214 (42) | 11 (22) |
| 500 | .. .. | 235 (47) | 30 ( 6) |
| 1000 | 301 (30) | 188 (19) | 12 ( 1) |
| 1000 | 315 (32) | 305 (31) | 36 ( 4) |
| 1000 | 454 (45) | 310 (31) | 92 ( 9) |
| 1000 | .. .. | 331 (33) | 101 (10) |
| Total | 1403/4020 (35) | 1780/5520 (32) | 348/5600 ( 6) |

In another series of investigations, 17 rabbits were given numbers of metacercariae ranging from 1 to 9 each, and sacrificed 1 month later. Those with fewer than 5 did not develop demonstrable infections. One of two given 5 metacercariae was found to harbor 1 adult worm, as also was one of two given 7. Both animals given 8 or 9 metacercariae were positive. The technic of pressing the liver between plastic plates appears to be quite effective in finding the worms, even when only a few immature ones are present. Thus it appears that the observations on the minimal infective dose are probably quite dependable.

Inocula of 10 and 1000 metacercariae were given to rabbits to determine whether percentage development and worm size were correlated with size of inoculum. The average number of worms recovered 20 days after 16 animals were given 10 metacercariae was 1.4 (14%) with mean size of $3.6 \times 1.1$ mm. Also at 20 days, 12 animals given 1000 metacercariae yielded an average of 73 (7.3%) worms per animal, with a mean size of $6.7 \times 1.4$ mm. The average number of worms recovered 40 days after 6 rabbits were given 10 metacercariae was 1.6 (16%) with mean size of $7.3 \times 2.1$ mm. Also at 40 days 6 animals given 1000 metacercariae yielded an average of 183 (18.3%) worms with mean size of $8.3 \times 1.9$ mm. Without regard to size of inoculum, the average size of worms taken from rabbits after 40 days was $7.9 \times 1.9$ mm.

*Susceptibility of snail intermediate hosts.* One hundred and fifty *Bulimus tentaculatus,* 75 *Gillia altilis,* 7 *Fluminicola coloradoense,* and 32 *F. fusca* were crushed and examined for the presence of sporocysts, rediae or cercariae. In no case were snails found to be infected although the feces of all 4 species at the time of examination contained numerous empty egg shells indicating that the eggs had been eaten and the miricidia liberated. Because the *F. coloradoense* were not adaptable to laboratory conditions, most died within a week. The results pertaining to this species should be considered accordingly.

## DISCUSSION

The metacercariae have been found to survive for long periods after death of the fish host. This makes possible shipments of metacercariae to this country for experimental work and also indicates that these fish can constitute a possible means of infection wherever they are imported for consumption in the U.S.A. It has been suggested by Binford (1934) that individuals in Hawaii may become infected in this manner.

Results of measurements of 500 living metacercariae indicate the average size to be $0.166 \times 0.148$ mm, which is smaller than was reported by Faust and Khaw (1927). Size variation, according to those authors, may be attributed to the growth of the metacercariae after encystation and the deposition of waste material between the larva and the wall of the cyst. However, their measurements are sufficiently larger to suggest other possible explanations. The likelihood that all measurements were not made with living material free from cover-slip pressure exists. Moreover, Hsü and Khaw (1936) indicated that the metacercariae described by Faust and Khaw were possibly not those of *C. sinensis.* Okabe (1937) reported that the described metacercariae may have been those of *Exorchis oviformis,* although their size ($0.13–0.24 \times 0.086–0.091$ mm) is still not within the range reported by Faust and Khaw.

It has been found that the guinea pig and rabbit are about equally susceptible to infection with *C. sinensis.* In these experiments metacercariae were introduced by dropper into the throat and it is reasonable to assume that a higher percentage may have developed if they had been administered by stomach tube. There appear to be definite host specificities since only 6% developed in rats while 60% developed in cats (Hsü and Wang, 1938). For laboratory experimental work, either rabbits or guinea pigs are good definitive hosts, although the rabbit offers the advantage of withstanding large numbers of flukes, and of providing a convenient source of serum over an extended period of time. It is well established that the cat is easily infected, but difficulty of handling, housing and caring for these animals makes their usefulness limited.

In 1923, Wayson reported that *Clonorchis* could not become established on the West Coast of the U.S.A. because, although many infected Orientals reside there, appropriate snail hosts evidentally are lacking in that region. The present work supports that view in that several species of the snail family Hydrobiidae were not susceptible to infections under the test conditions.

## SUMMARY

Metacercariae of *Clonorchis sinensis,* shipped under refrigeration from Okayama, Japan, to the U.S.A., retained their viability for periods of at least 60 days

after death of the fish host. Rabbits and guinea pigs were found to be equally susceptible to infection with this parasite, about one-third of the orally administered metacercariae developing to maturity. Rats were less susceptible, with only 6% development. An inoculum of 1000 metacercariae was lethal to guinea pigs, but rabbits were able to withstand even larger inocula.

Egg production was detected an average of 22 days after infection in rabbits, and after an average of 20 days in guinea pigs. It was found that as few as 5 metacercariae given by mouth were capable of producing demonstrable infections in rabbits. Approximately the same percentage of development was found with inocula of from 10 and 1000 metacercariae. The average size of adults recovered did not appear to be influenced by the number of worms present.

Attempts to infect snails of the family Hydrobiidae (*Fluminicola coloradoense, F. fusca, Gillia altilis,* and *Bulimus tentaculatus*) with *C. sinensis* were unsuccessful, but since it was not possible to maintain *F. coloradoense* in the laboratory, further studies may be required to determine whether this species is susceptible to experimental infection.

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## RESEARCH NOTE

### *CORRODOPSYLLA CURVATA CURVATA* (ROTHSCHILD) (SIPHONAPTERA) IN WASHINGTON

During 1953–1956, series of small wild mammals were collected in eastern and central Washington for the purpose of testing their tissues and ectoparasites for pathogenic bacteria. This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171 and in part by the Office of Naval Research as Project NR 135 180, Contract Nonr 1007 (00) to the junior author of this paper. The collection included some shrews together with their fleas. The following new location records were obtained.

From 2 *Sorex vagrans* Baird taken from a streamside 4 miles west of Davenport, Lincoln County, August 9, 1953, 1 male and 1 female *Corrodopsylla curvata curvata* (Rothschild), preserved and retained; 2 males, determined as *C. c. curvata* by examination of genitalia, were triturated for bacteriological tests.

From 2 *Sorex vagrans* taken from the same location, June 30, 1954, 3 males, 1 female *C. c. curvata*, preserved and retained; 12 males, determined as *C. c. curvata* by examination of genitalia, and 7 females, identified as *Corrodopsylla* sp., were triturated.

Two fleas were taken from the bag in which 1 *Sorex vagrans* together with several deermice, *Peromyscus*, had been collected on Cloverland Grade, Asotin County, February 20, 1955, G. E. Hudson and W. L. McNeil collectors. These fleas were determined by examination of the genitalia as *C. c. curvata* males and later were triturated.

Other specimens of *Sorex* totaling 13 *S. vagrans* and 5 *S. merriami merriami* Dobson were found to be free from siphonapteran parasites.

Hubbard (1947. Fleas of Western North America. Iowa State College Press, Ames, Iowa, 533 pp.) gave as the probable ranges of the true shrew fleas of western North America "everywhere east of Great Basin" for *Doratopsylla* (*Corrodopsylla*) *curvata curvata* and "west of Great Basin to Pacific Ocean" for *Doratopsylla jellisoni* Hubbard (*Corrodopsylla curvata obtusata* (Wagner)). He recorded *D. jellisoni* from locations west of the crest of the Cascade Mountains in Oregon and Washington and *D. c. curvata* from Ravalli County, Montana.

The zone of transition between the ranges of the 2 groups of fleas in the United States is unknown. Aridity probably excludes shrew populations from some areas of eastern and central Washington, but there are probably other areas where shrew populations have a continuity from east to west across the state. The records of Holland (1949, The Siphonaptera of Canada. Dominion of Canada, Dept. Agr. Publ. 817, Technical Bulletin 70, 306 pp., 142 pls.) suggest that, in British Columbia, the crest of the Cascade Mountains separates the ranges of *Corrodopsylla c. curvata* and *C. curvata obtusata*. Likely this transition line is continuous with the Cascade Range into Washington although concrete evidence in the form of collection records is still lacking.—MARION BACON* AND CHARLES H. DRAKE, *Department of Bacteriology and Public Health, State College of Washington, Pullman, Washington.* (* Present address: Department of Microbiology, University of Nebraska College of Medicine, Omaha, Nebraska.)

# OCCURRENCE OF LARVAL TREMATODES IN THE COLUMBIA BASIN*

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The data presented herein are the results of a study of the larval trematode infections of fresh water snails of the Columbia Basin, Washington, from the spring of 1956 through the spring of 1957. The present study is the first of its kind in the Columbia Basin. Preliminary information concerning the larval trematodes of this area was reported in 1957 by the authoress under her maiden name of A. Deforest.

Grand Coulee Dam on the Columbia River impounds Roosevelt Lake, the water of which irrigates the arid lands composing most of the "Big Bend" area in the Columbia River Basin. Water which is in excess of irrigation needs, water which has traversed the fields to the north, west, and east of Moses Lake, and water from the blocked channel of Crab Creek is impounded behind O'Sullivan Dam, a 4-mile earth and stone structure which was constructed by the United States Bureau of Reclamation during the 1946–52 period. Potholes Reservoir, which resulted following the completion of O'Sullivan Dam, covers nearly 20,000 acres and has a depth in excess of 100 feet. As a result of water seepage through the dam, many new, permanent, seep ponds and seep lakes were formed. These have been used by migratory waterfowl for nesting sites and planted with various species of game fish by the State of Washington Department of Game. The present study was undertaken to determine the prevalence of larval trematode infections among the fresh water snails in various habitats in the area, and to identify the trematodes as far as major groups.

## METHODS

Collections were made from the following types of habitats:

*Seep ponds*—The term "seep pond" refers to any of the many small bodies of water which are the result of the completion of O'Sullivan Dam and the formation of Potholes Reservoir. Seepage through the dam and banks of the irrigation canals formed permanent bodies of water, with abundant aquatic flora and fauna.

*Seep lakes*—The seep lakes are larger permanent bodies of water also formed as the result of seepage through O'Sullivan Dam. The aquatic life found in the seep lakes is similar to that found in the seep ponds.

*Crab Creek*—This stream was present in the area for many years before the irrigation project began, and is the oldest body of water from which snails in this study were collected.

*Irrigation canals*—The irrigation canals vary greatly in size and rate of water flow, but the quality of water appears to be quite constant throughout the irrigation system. Snails were examined from both main irrigation canals and from lateral irrigation canals which deliver water from the equalizing reservoirs to the irrigated fields.

*Wasteways*—The wasteways contain runoff water and water which is shunted from an irrigation canal. As a result, these waters are not uniform as to rate of flow, turbidity, or temperature.

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The writer wishes to express her sincere appreciation to Dr. Waldemar M. Walter for identifying the snails used in the study and for aiding in the collection of material, and to Dr. Charles W. McNeil, under whose direction the work was done, for his interest and constructive criticisms.

Two species of snails were examined, namely, *Physa propinqua* Tryon, 1865, and *Stagnicola palustris nuttalliana* (Lea, 1841). Field-collected snails were brought into the laboratory and isolated in wide-mouth specimen jars. The technique followed in the examination of the snails was similar to that used by previous workers (Harper, 1929; McCormick, 1923; Cort, 1915; Miller, 1926; Stunkard, 1930). If mature cercariae were present they usually emerged from the snail within 24 hours after isolation. Cercariae were examined microscopically at once and the snail kept alive as a future source of study material. If a snail was not shedding cercariae the body was crushed and dissected in water in a watch glass. The exact percentage of infection was determined by crushing all snails from which cercariae were not emerging after 24 hours' isolation. These snails yielded information on the presence of metacercariae and evidences of mixed infections.

## OBSERVATIONS

Table I shows the major types of habitat from which collections were taken, the

TABLE I.—*Infections by locality and snail hosts.*

| Habitat type | *Stagnicola palustris nuttalliana* | | | *Physa propinqua* | | |
|---|---|---|---|---|---|---|
| | Exam. | Pos. | % | Exam. | Pos. | % |
| Seep ponds | 889 | 495 | 55.7 | 282 | 64 | 22.7 |
| Seep lakes | 362 | 326 | 90.1 | 9 | 4 | 44.4 |
| Crab Creek | 473 | 47 | 9.9 | 12 | 1 | 8.3 |
| Main irrigation canals | 19 | 1 | 5.3 | 756 | 27 | 3.6 |
| Lateral irrigation canals | 447 | 9 | 2.0 | 264 | 6 | 2.2 |
| Wasteways | 46 | 7 | 15.2 | 7 | 0 | 0.0 |
| Total | 2236 | 885 | 39.6 | 1330 | 102 | 7.8 |

number of snails of each species examined, the number and the percentage of snails parasitized. Table II shows the major groups of larval trematode parasites found

TABLE II.—*Cercarial type and host locality.*

| | Seep ponds | Seep lakes | Crab Creek | Main irrig. canals | Lateral irrig. canals | Waste-ways |
|---|---|---|---|---|---|---|
| Monostome | S—P | | S | | S | S |
| Echinostome | S | | | P | | |
| Xiphidiocercaria | S—P | S—P | S | S | S | |
| Xiphidiocercaria with fin-fold | S | | | | | |
| Leptocercous cercaria | S—P | S | S | P | P | |
| Furcocercous | S | S | S | | | |
| Metacercaria | S—P | S—P | S—P | P | S—P | S |
| Sporocysts and rediae | S—P | S—P | S | P | S | |

S—*Stagnicola palustris nuttalliana* (Lea, 1841).
P—*Physa propinqua* Tryon, 1865.

in the 2 species of snails in each of the major types of habitat. Table III shows the major groups of larval trematode parasites and their prevalence. It should be noted that Tables II and III indicate that furcocercous cercariae were present. However, both apharyngeal brevifurcate distome furcocercous cercariae and pharyngeal longifurcate distome furcocercous cercariae are included in this one category. The exact number of snails infected with each of these types is not available.

The presence of sporocysts and rediae appeared to injure the digestive glands of the snail hosts. In many cases the digestive gland was slightly enlarged and mottled, with rediae visible near its surface. The encysted stages of cercariae appeared to do very little harm to their snail hosts even when present in large numbers.

These observations are in agreement with those of Lebour (1912) and Harper (1929). A few snails collected from the seep ponds and seep lakes showed degeneration of the liver which could be attributed to previous trematode infections, but no parasites were present in the organ.

Table III shows that an encysted metacercaria was the most common form found in the area. This metacercaria was widespread in snails collected from Crab Creek, the seep ponds, the seep lakes, and the irrigation canals.

As can be seen from Table I, the seep ponds and seep lakes had by far the highest percentage of infected snails. The irrigation canals showed a much lower rate of infection. The explanation seems to be that the snails in the usually calm ponds and lakes had a much greater opportunity to become infected than those in the irrigation canals. The rapid flow of water in the irrigation canals lessens the chances

TABLE III.—*Cercarial type and host infection.*

| | *Stagnicola palustris nuttalliana* | | *Physa propinqua* | |
|---|---|---|---|---|
| | Number infected | Percentage infected | Number infected | Percentage infected |
| Monostome | 10 | 0.4 | 1 | 0.1 |
| Echinostome | 4 | 0.2 | 1 | 0.1 |
| Xiphidiocercaria | 73 | 3.2 | 8 | 0.6 |
| Xiphidiocercaria with fin-fold | 2 | 0.1 | 0 | 0.0 |
| Leptocercous cercaria | 7 | 0.3 | 5 | 0.4 |
| Furcocercous | 20 | 0.9 | 0 | 0.0 |
| Metacercaria | 761 | 34.0 | 82 | 6.2 |
| Sporocysts and rediae | 8 | 0.4 | 5 | 0.4 |
| | 2236 Examined<br>885 Infected | | 1330 Examined<br>102 Infected | |

of contact between parasite and host throughout the life cycle of the parasite. Multiple infections, where they occurred, were also found in the seep ponds and seep lakes, again indicating the much greater chance for snails to become infected with larval trematodes of different species in the areas where a wide variety of definitive hosts exist.

## SUMMARY

1. A total of 3,566 snails of 2 species, *Physa propinqua* and *Stagnicola palustris nuttalliana*, was collected from the Columbia Basin area of Washington during the 12-month period, April 1956–April 1957, and examined for larval trematodes. Of these, 987 snails, or 27.7%, were parasitized with larval trematodes.

2. Cercariae were placed in major groups as follows: monostome cercariae, echinostome cercariae, xiphidiocercariae, xiphidiocercariae with a fin-fold, leptocercous cercariae, apharyngeal brevifurcate distome furcocercous cercariae, and pharyngeal longifurcate distome furcocercous cercariae.

3. An encysted metacercaria was the most common parasite found.

4. The seep ponds and seep lakes showed the highest percentage of infected snails, while the irrigation canals had the lowest infection rate.

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## RESEARCH NOTE

### *PARAGONIMUS* IN WILD AND DOMESTICATED ANIMALS IN GEORGIA

In April, 1957, Drs. Dennis Sikes and Frank A. Hayes, Department of Pathology and Parasitology, School of Veterinary Medicine, University of Georgia, brought into the laboratory a recently killed bobcat, *Lynx ruffus*. The bobcat had fallen dead or had been discarded alongside State Route No. 15, just over the line in Hancock County, Georgia. Its body carried 1 rifle slug, and the flesh was still relaxed when the animal arrived in the laboratory. Two specimens of *Paragonimus kellicotti* were removed from the diphragmatic lobe of the left lung; they occupied the same cyst. One of the worms was teased to pieces to show its internal structures as a class demonstration. The second worm was flattened under coverslip pressure, stained in Bullard's hematoxylin and mounted in Balsam. It measured 14.80 mm long by 6.60 mm in maximum width. The oral sucker was about 1.10 mm in diameter and the acetabulum was slightly narrower than long, measuring 0.95 mm wide by 1.00 mm long.

Insofar as the writers know this report records the first and only authentic case of *Paragonimus* from a wild host in the State of Georgia. It is to be pointed out that the bobcat had been shot by an unknown hunter at some hour earlier than that at which the body was found and that the wounded animal could have traveled a considerable distance between the time it had been shot and the time of death. It seems quite unlikely, however, that the wounded animal could have covered the 45 miles between its location when found and the State of South Carolina, the other nearest State from which the cat might possibly have come. This assumption is based on the proposition that some hunter did not discard the animal at this point after hauling it for several miles. Further, it is to be pointed out that Cooperrider (1952, Vet. Med. **47**: 65–70) recorded *Paragonimus* from the lungs of pigs, *Sus scrofa*, in Georgia. In a personal communication under date of March 11, 1958, Dr. Cooperrider states: "I do remember that several specimens were found in swine, in lung abcesses, at autopsy during my first year at Georgia (1949–50). In addition, during that same year or the next, several specimens were found in hog lungs brought from White Provision Company in Atlanta." In searching the necropsy records in the School of Veterinary Medicine, 2 cases of paragonimiasis, with substantiating sections of flukes in lung cysts are present for the year 1950. These represent the "hog lungs" from the Atlanta area mentioned above. No other record of *Paragonimus* has been found. Thus, the question of origin of the infected pigs is in doubt since there is no indication as to the section of the country from which these pigs came to slaughter.

In a personal communication from Mr. Allen McIntosh, dated March 12, 1958, it is pointed out that a specimen, No. 49428, of *Paragonimus* "from hogs from Fitzgerald, Georgia, 4-30-54" is on deposit in the Helminthological Collection, United States National Museum. Mr. McIntosh states: "Most of these records are from hogs from slaughter houses, but the localities from which the hogs were shipped are not given in most cases."

During May, 1954, Dr. C. A. Blount, Jr., of Waynesboro, Georgia, forwarded to the School of Veterinary Medicine the lungs of a 1-year-old pointer dog. At least 4 cysts were found in the lungs, and each contained flukes identified as belonging to the genus *Paragonimus*. The flukes had been removed from 1 of the cysts by Dr. Blount, and placed in a separate container. Although these flukes were included in the shipment, the freed specimens were lost through damage to the package in transit. The remaining 3 cysts were embedded in paraffin and sectioned. Each cyst contained 2 mature *Paragonimus*, lying "face to face." The dog is of unknown origin although when killed it resided in Georgia and had been under observation for about 1 week. It is recorded that the dog's temperature was normal and that there was "labored breathing 'as seen in severe pneumonia or ruptured diaphragm.'"—Helen E. Jordan, *Department of Pathology and Parasitology, School of Veterinary Medicine,* and Elon E. Byrd, *Department of Zoology, School of Arts and Sciences, University of Georgia.*

# TISSUE RESPONSE TO INFECTION WITH *CONSPICUUM ICTERIDORUM* (TREMATODA), IN THE GALL BLADDER OF THE BREWER BLACKBIRD

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Although reports of pathology attributed to liver flukes are numerous (Faust and Khaw, 1927; Hoeppli, 1933; Craig and Faust, 1951; Hou, 1955, 1956), most of these present the clinical effects of fluke invasion in man and very few (Hoeppli, 1933; Hou, 1955, 1956) have discussed the histopathology as such.

Examination of the gall bladders of 70 Brewer blackbirds, (*Euphagus cyanocephalus*), in southern California showed that well over 50% of them were infected with a dicrocoeliid fluke, ***Conspicuum icteridorum*** Denton and Byrd, 1951.

These flukes attach themselves to the lining of the gall bladder by means of a powerful muscular ventral sucker. When the worms are removed from the gall bladder, the epithelium remains elevated in mushroom-like tufts which are readily visible with the unaided eye (Fig. 1).

Microscopical examination of both non-infected and infected gall bladders indicates that marked histological changes occur in the structure of the gall bladder in infected birds.

## MATERIALS AND METHODS

Serial sections of 16 infected and 7 non-infected gall bladders were cut at 7 microns.

Zenker-formol and Bouin's solutions were used as fixatives. Generally the gall bladders were fixed in toto along with the contents, immediately after decapitation of the birds.

Harris' hematoxylin and eosin stain was used for general survey of the tissue. Masson's trichrome and Mallory's triple stains were employed for the demonstration of connective tissue in general and Weigert's stain for elastic tissue.

## OBSERVATIONS

The most striking change in the gall bladder is the alteration in the thickness of the wall of the organ (Fig. 2). When compared with the non-infected bladder the mucosa of which is thrown into deep folds (Fig. 3), usually the wall of the infected gall bladder gives the appearance of a thin line and there is a definite loss of supporting tissue and wall substance. It is recognized that the thinning of the wall is probably due in part to pressure brought about by the bulk of the worms added to the normal amount of bile. However, upon close examination actual structural changes in the wall can be seen. For example, in many instances, the whole of the epithelial layer has been sloughed off so that no epithelial cells are visible. In some cases, even the lamina propria is gone and the surface (lumen) layer is that of the muscularis (Fig. 4). Some of the desquamated tissue can be seen as debris in the lumen. The muscle layers are stretched. The dense connective tissue usually seen surrounding the bundles of longitudinal muscle in cross section is completely absent.

At the points of attachment of the worms to the epithelium, the mucosa shows the greatest change. Here, in striking contrast to the other regions, the wall is thickened tremendously, so that it appears as a mushroom-like projection many times the

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size of the free wall (Fig. 5). These projections, or mushroom-like tufts, in no way resemble the villus-like folds of the normal, non-distended bladder. The tuft is broad with a shape resembling a "button" mushroom. There is no definite columnar epithelium. In many instances the free surface of the tuft is broken, giving the appearance of sloughing.

In the tuft, there is considerable proliferation of connective tissue. This tissue in proliferating appears to have completely displaced pre-existing muscle bundles. The elastic fibers which make up a good proportion of the growth, radiate out from the narrow or "stalk" end of the mushroom and extend to within a very short distance of the rounded, upper surface which comes in contact with the lining of the sucker (Fig. 6). In addition, tubular, gland-like structures lined with cuboidal cells occur within the body of the tuft (Fig. 6). No blood components such as white blood cells, which might indicate an active inflammatory process, can be seen. In fact, capillaries which normally are found in the lamina propria, are absent and the usual layers of the epithelial wall cannot be identified. Rather it appears that all the usually definable regions have been replaced by collagenous and elastic fibers.

In some infected gall bladders, necrotic tissue, in large amorphous clumps, lies adjacent to well-developed tufts, and may also be seen in areas of adenomatous tissue formation where the latter has developed at the expense of the normal gall bladder epithelium (Fig. 7).

In still other areas where tufts have not developed, a granulomatous, inflammatory swelling has been noted in close proximity to a fluke (Fig. 8). Here, many large swollen macrophages can be seen and lymphocytes are in abundance. There are numerous blood vessels, the walls of which, in many instances, are edematous. In the region of the swelling, the continuity of the muscle layers of the wall is interrupted as if the fibers had been split apart by the growing inflammatory nodule, and the broken ends can be seen in adjacent areas apparently not involved or affected by the inflammatory process.

## DISCUSSION

Hoeppli (1933) in his discussion of tumor development in relation to *Opisthorchis felineus* and *Clonorchis sinensis* reported inflammation of the bile ducts followed by thickening of the wall due to elaboration of connective tissue. He found an increase in the periportal (interlobular) connective tissue which led, in some cases, to periportal cirrhosis. Proliferation of the duct epithelium was also observed; this, Hoeppli felt, was especially important in connection with development of carcinoma.

Hou (1955, 1956) found an abundance of adenomatous tissue growth and little connective tissue in heavy infections of short duration and the reverse in chronic cases. He attributes at least 15% of human carcinoma of the liver in Hong Kong to this fluke but sees no direct relation between clonorchiasis and multilobular cirrhosis.

Tissue response to liver flukes in vertebrates besides man, in general, has been ignored. Smith, Coates and Nigrelli (1941) found papillomatous growth in association with *Rhytidodoides similis* Price 1939 (Rhytidodidae Odhner 1926) in the gall bladder of the marine turtle, *Chelonia mydas*. These outgrowths were reported as a hyperplasia of the mucous membrane. The hyperplastic areas far exceed the size of the whole worm and appear to be a response of the host tissue to the presence of the flukes but do not appear to be related to any particular part of the worm, such as the ventral sucker.

Mapes (1951) during his extensive investigation into *Dicrocoelium dendriticum* infection of sheep observed a definite histopathology of the periphery of the liver and the intrahepatic ducts. Peripheral sclerosis was found accompanied by a fibrous thickening of the walls of the bile ducts, glandular proliferation and a later replacement of hepatic cells by collagenous tissue fibers.

Experimentally induced fascioliasis in laboratory rabbits (Urquhart 1956) produced histological changes such as connective tissue proliferation of the liver parenchyma in the areas of migration of the worms leading to a cirrhosis, as well as extensive fibrosis of the bile ducts occupied by the worms.

Kulasiri and Seneviratne (1956) report a monolobular cirrhosis with dilated bile ducts, atrophied discontinuous strands of smooth muscle with fibrous tissue between the strands, as well as liver parenchymal infiltration of round cells in liver infestation with *Gigantocotyle explanatum* (Paramphistomidae) of the buffalo. A passing reference is made to a "flask-shaped projection" of the mucosa which filled the cup of the posterior sucker but no statement is made of the histology of this area.

Timon-David (1953) mentions damage produced by the dicrocoeliid *Brachylecithum lobatum* in the bile ducts of the magpie *Pica pica*. Since as many as 6 flukes are found in a single duct the changes are mainly destruction of tissue due to pressure, with some thickening of connective tissue. No histological alterations are pictured. Leucocytosis is marked.

Recently, Jiménez-Quiros and Brenes (1957) have described some histological changes in the bile ducts of the howler monkey, *Alouatta p. palliata* brought about by the dicrocoeliid *Controrchis caballeroi*. The small bile ducts showed an increase in the pericanicular collagen. The epithelium of the increase in the pericanicular collagen. The epithelium of the bile ducts sometimes showed a mild adenomatous hyperplasia.

Invasion of the Brewer blackbird gall bladder by *C. icteridorum* elicits a very definite response on the part of the bird host which in part can be correlated with mechanical activity of the ventral sucker of the worm.

It appears that the results of fluke invasion can be summarized under 2 broad headings:

(1) Acute stage (Fig. 9)—the response of the host organism to invasion, including the familiar inflammatory reaction of living tissue to damage, followed by a healing period during which connective tissue is elaborated.

(2) Second stage—this stage can be sub-divided as follows: (a) Chronic, and (b) malignant.

The chronic stage (Figs. 5 and 6) may be permanent and is the result of the continued ability of the host tissue to heal despite the presence of the flukes, and may be considered as a state of equilibrium established between the host tissue and the worms. In this investigation this is characterized by the tufts made up almost entirely of connective tissue (Fig. 5).

Adenomatous tissue growth (Fig. 7) would seem to be a host response going well beyond this chronic stage, and is evidence of the inability of the tissue to maintain metabolic equilibrium under continued irritation. It was found infrequently.

## SUMMARY

Histological changes which take place in the gall bladder of the Brewer blackbird in the presence of *Conspicuum icteridorum*, a dicrocoeliid fluke, are localized connective tissue hyperplasia, loss of wall substance and supporting tissue, as well

as, in some instances, the presence of necrotic tissue, inflammatory processes and the development of adenomatous tissue.

Brewer blackbird tissue response resembles that of other hosts (i.e. sheep and rabbits) to fluke invasion and can be considered as occurring in 2 major stages, acute and chronic. These 2 stages can be correlated with the duration of the infection.

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## Explanation of Plates

### Plate I

Figure 1. Lining of gall bladder showing tufts formed by ventral sucker of worm. Fresh tissue, unstained. ×20

Figure 2. Whole infected gall bladder. Note thin wall (W). Mallory III stain. ×25

Figure 3. Whole non-infected gall bladder. Hematoxylin and eosin stain. ×25

Figure 4. Infected gall bladder. Note desquamation and reduction of wall. Mallory III stain. ×100

Figure 5. Tuft (T)—greatly enlarged area of gall bladder wall held within the ventral sucker (SU) of the worm. Mallory III stain. ×100

Figure 6. Tuft of gall bladder wall within ventral sucker of worm. Note tubular, gland-like structures (TG). Elastic tissue fibers can be seen as dark lines within the tuft. Weigert elastic tissue stain. ×100

### Plate II

Figure 7. Infected gall bladder showing necrotic tissue (NT) and adenomatous tissue growth (ADT). Hematoxylin and eosin stain. ×50

Figure 8. Inflammatory nodule. Note fluke (F) in close proximity and break in muscle layer (M). Mallory III stain. ×50

Figure 9. Infected gall bladder showing beginning tuft formation and presence of leucocytes in the area. Hematoxylin and eosin stain. ×100

### Abbreviations for Plate Figures

ADT, Adenomatous Tissue; D, Duct; F, Fluke; IN, Inflammatory Nodule; M, Muscle layer; NT, Necrotic Tissue; S, Serosa; SU, Ventral Sucker of Worm; T, Tuft; TG, Tubular, gland-like structure; W, Wall of Gall bladder.

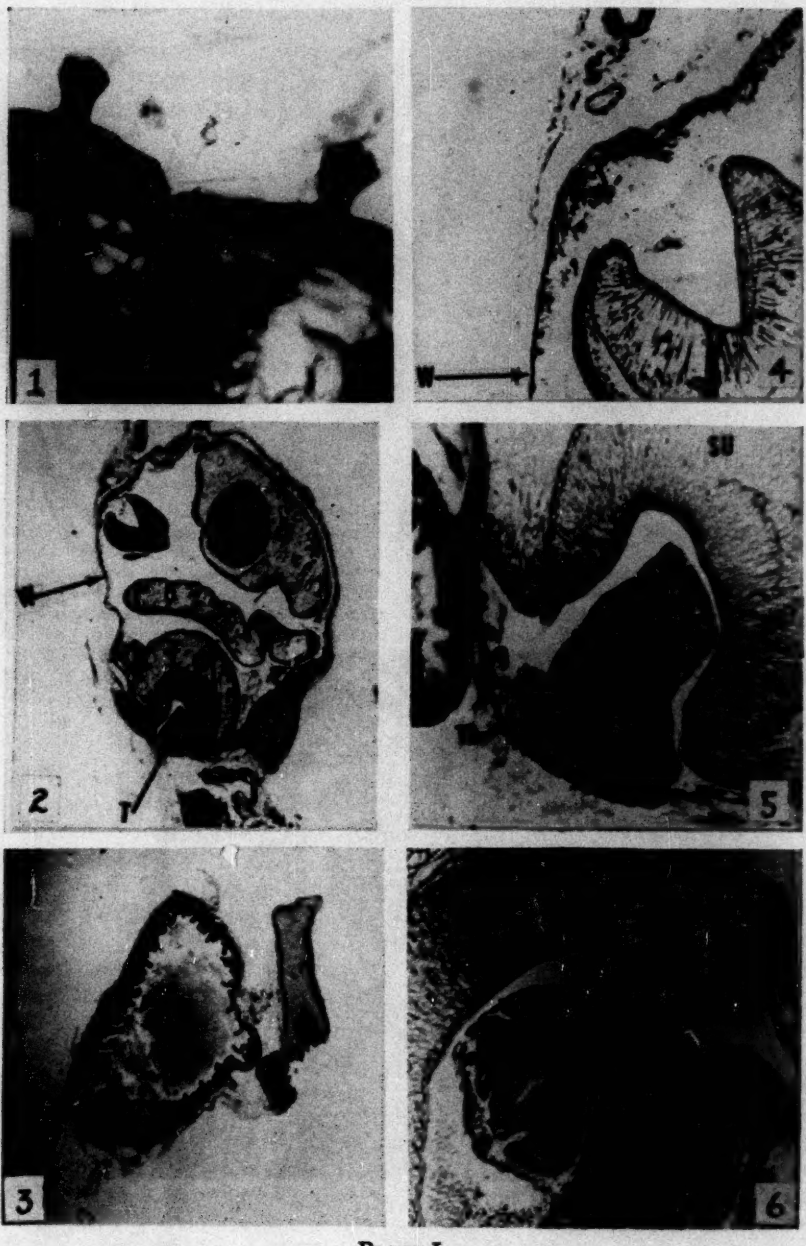
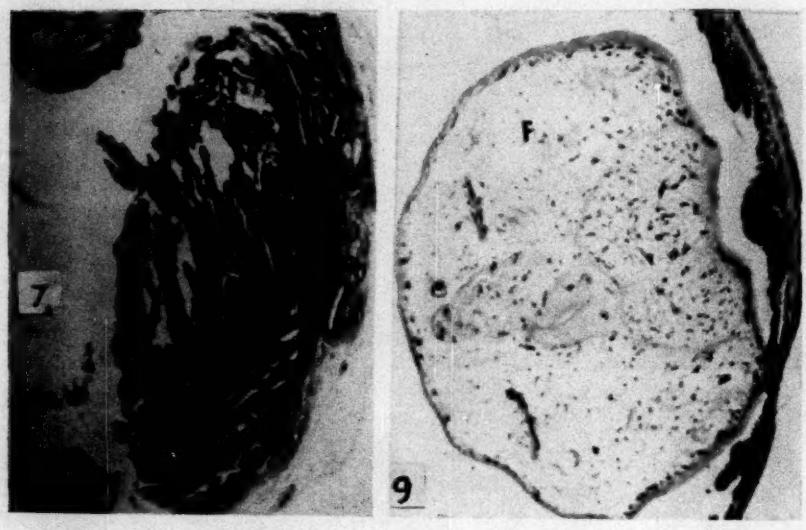



PLATE I



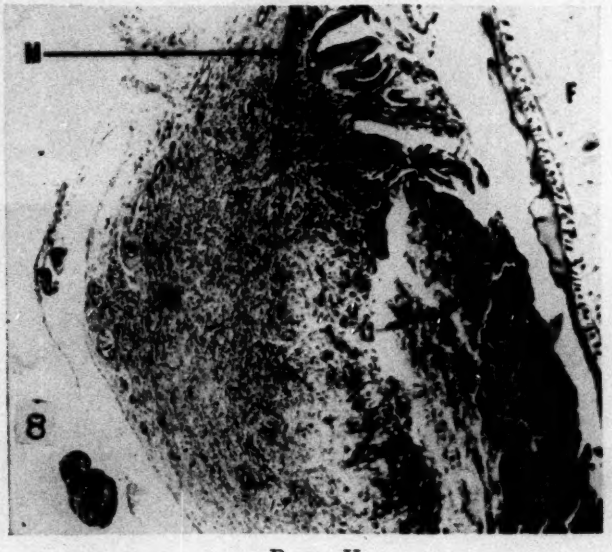




PLATE II

# THE HEXACANTH EMBRYO OF A DILEPIDID TAPEWORM
## I. THE DEVELOPMENT OF HOOKS AND CONTRACTILE PARENCHYMA

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It has been of considerable interest for the writer to learn that oncospheres are not all produced in the same way. The kind of embryo and the manner of its production are related to taxonomic position of the genus. In the present series of papers the morphology and development of a typical dilepidid hexacanth embryo will be described as a probable pattern for certain genera of the family Dilepididae. The complete hexacanth embryo (Fig. 18) was an oncosphere which retained the embryonic epidermis that later developed prominent epidermal glands (Reid, 1948; Ogren, 1955, 1957).

### MATERIAL AND METHODS

The cestode species used was identified as *Dilepis undula* (Schrank) from the small intestine of the robin, *Turdus migratorius*, collected in Champaign-Urbana, Illinois. The cestode was freshly killed, without prolonged bathing in mammalian Ringer's solution, by flooding with Carnoy fixative. The specimen was stored in Carnoy, then transferred to absolute alcohol, and serially sectioned as previously described (Ogren, 1955). Stains employed were those used in previous studies: van Gieson's connective tissue stain, metachromatic stain of Lillie (1954) using Weigert's acid hematoxylin and safranin O, allochrome periodic Schiff reaction, toluidine blue, methyl green-pyronin, hemalum and orange G, aniline acid fuchsin, Delafield's hematoxylin and eosin. Malachite green and bromphenol blue were employed as suggested by Johri and Smyth (1956) for determination of basic proteins.

### OBSERVATIONS

The hexacanth embryo had the appearance shown in Figure 18. This oncosphere was enclosed by an outer rigid shell and an inner colloidal cushion or pseudoembryophore derived from oncosphere secretions (Fig. 19). The active body of the hexacanth embryo was enclosed by a thin cuticle and was composed of a contractile parenchyma. In the living condition cell boundaries and nuclei were difficult to identify and the appearance of the body was that of an homogeneous contractile protoplasm. Three characteristic pairs of hooks were present. Hooks were located in only 1 hemisphere and were regularly moved. Prominent granular areas were present that represented epidermal glands, or the penetration glands of Reid (Fig. 18).

#### *The Morula*

Cleavage of the zygote occurred following polar body formation. One group of blastomeres containing large amounts of yolk substance was excluded from formation of the sphere of blastomeres that produced the morula (Fig. 11). These were considered to be products of the secondary macromere and will be explained in another paper. A thin capsule, produced by shell substance in the uterus, enclosed the morula. The earliest sign of differentiation was the grouping of cells at the epidermis. At this time the blastomeres were easily recognized and had abundant granular cytoplasm that stained red with pyronin and purple with toluidine blue.

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The cortical cells tended to multiply and produce a syncytium. Continued divisions of certain cells resulted in nuclei of different sizes (Figs. 11, 12). The appearance of 3 prominently stained cell areas marked the beginning of hook, medullary contractile center, and epidermal gland formation (Figs. 12, 19). Epidermal cells of the embryo failed to detach and were retained. Certain of these cells became epidermal glands (Figs. 12, 13).

### *The Hooks and Their Formation*

Each hook (Figs. 1–3) consisted of a curved blade, a thickened collar (guard) and a straight shank (handle). The 3 pairs were arranged in a characteristic manner (Fig. 18). The small slender lateral pair (Fig. 1) was more anterior and dorsal; the large clumsy pair was more ventral and lateral (Fig. 2). The medial hooks (Fig. 3), were very slender. Their thin blades curved toward the substrate and for this reason were difficult to represent in the same plane with the other hooks (Fig. 8).

Hooks developed in prominently stained granular oncoblasts in the cortex of the morula (Fig. 13). They were the earliest structures to differentiate (Figs. 11, 12). Usually the 2 lateral hook blades appeared first (Figs. 5, 13) and thus formed convenient land marks during study. The blade was secreted inside the cell (Fig. 5) and appeared as a round, brightly colored mass in transverse section (Fig. 6). Shank development also took place in the cell (Fig. 4) but it was not learned whether this was the same cell or a different cell from the one that formed the blade. The writer believes it may be the same cell.

Oncoblasts remained attached to the shank when hook development was completed. Developing hooks stained red with safranin O, purple with toluidine blue, yellow with van Gieson's connective tissue stain, red with aniline acid fuchsin, green with bromphenol blue. They also showed positive reactions for proteins by the xanthoproteic, Millon and ninhydrin Schiff reactions. It has been suggested by other investigators that the hooks are scleroprotein in nature.

### *Parenchyma and Muscle*

One problem that has interested the writer for some time is that of explaining the cellular basis for movements of the oncosphere. The body of the oncosphere is composed of a loose mesenchyme that is contractile. The problem is related to a study of mesenchyma in general. The cells developed a cortical parenchyma that was associated with constrictions of the body and extension of the hooks. A medullary contractile center formed a contractile system associated with shortening of the body and retraction of hooks. Certain observations on these 2 regions will be presented below, as well as on fiber attachments to the hooks. Additional problems to consider are: 1) The nature of the intercellular substances, sources for these materials, and direction of diffusion; 2) Origin of fibrils, from the cell or outside the cytoplasm; 3) The chemical nature of the fibrils: collagen, myosin; 4) Is the cytoplasm of the cell contractile, or only the fibers?

The cortical parenchyma appeared to develop as follows. In the morula cortical cells beneath the thin cuticula were arranged in a syncytial manner with cell boundaries indistinct (Fig. 10). The large spherical nucleus was enclosed by a small amount of granular cytoplasm. The cortical cells continued their embryonic cell divisions until their nuclei were the diameter of small parenchymal nuclei of the

adult tissue. Thus the small cortical or "somatic" nuclei arose (Figs. 9, 14, 19). Careful study of these small nuclei in early oncospheres (Figs. 11, 12) demonstrated that they were enclosed by a finely granular, yet fibrous cytoplasm. The cell boundaries were indefinite, but certain isolated cells had the appearance shown in Figure 15. Delicate fibers arose from the cytoplasm and passed to other cells or to the cuticle. Varying with the fixative used, fibrils were sometimes seen in the cytoplasm. As the oncospheres became older, changes occurred in the cortex to produce a much thicker cuticle and more fibers. The later condition of cortical cells can only be briefly described. The nuclei were enclosed by a very thin region of protoplasm as diagrammed in Figure 16. The nucleus stained dark and numerous fibers associated the cell with other cells (Figs. 9, 14). Some nuclei were very small, shrunken and more darkly stained because of a thick nuclear membrane. In older oncospheres there was a complex abundance of fibers. The associations of fibers probably vary somewhat with the individual oncosphere. In old oncospheres contractile substance became so abundant that individual fibers were not recognized. Thus younger oncospheres were selected for study. These observations emphasize the importance of studying the development of the oncosphere in order to interpret the completed structure.

The medullary contractile center, first recognized in sections of *Hymenolepis nana*, was described as a binucleate primordium (Ogren, 1955). It was later considered to be a medullary contractile center of *Oochoristica* (Ogren, 1957). The medullary contractile center in *Dilepis* was found in early oncospheres before hooks developed, as a binucleate primordium (Figs. 11, 12). The cells were large. The cytoplasmic granules stained prominently with pyronin, or toluidine blue. The area became multinucleate during subsequent development but the cells continued to be prominent (Figs. 13, 14). The medullary contractile center appeared to be responsible for the formation of intercellular material (Figs. 17, 18) of a contractile nature. Fibers from the medullary center made complex associations with cortical fibers and with the hooks (Figs. 8, 14, 18). Following completion of the hooks abundant fibers from the center developed throughout the oncosphere. The above observations demonstrated that the body of the hexacanth embryo made a series of transformations from the embryonic mesenchyma to a contractile parenchyma. Cortical and medullary fiber systems were present to account for body and hook movements.

### *Hook Musculature*

Fiber attachments to the hooks were complex and only a few were described. The greatest amount of muscle was concentrated in the hemisphere with the hooks. Hooks were fixed in position at the union of the collar and the cortex. The lower part of the collar on either side was enlarged and formed 2 blunt barbs, or projections (Fig. 7), to which heavy muscle strands were attached (Figs. 8, 9, 18).

Muscle strands attached to the barbs and collar were derived from special cells in the cortex near the hook, and were firmly attached to the cuticle (Figs. 7, 8). The fibers served to pull the hooks outward and back and were visible in life. Parenchymal muscle fibers from adjacent myoblasts were also attached to the straight part of the hook (Fig. 9). The terminal ends of the lateral hooks were bound to muscle fibers from the medullary contractile center (Figs. 9, 18) and the cortical parenchyma.

The slender medial hooks were slightly ventral in position. Muscle fibers bound the hook to the cuticle by attachment to the collar (Fig. 7). Their most frequent movements were downwards toward the substrate. Muscle fibers attached to these hooks were complex in arrangement and tied into the parenchyma (Fig. 8). The terminal ends of the medial-hook-shanks were associated with numerous fibers from the contractile center (Fig. 8) and other fibers of the parenchyma (Fig. 7).

In the forward part of the oncosphere there were a few long circular or transverse fibers derived from several myoblasts (Fig. 18). They were associated with hook muscle fibers and were among the largest fibers stained. In living oncospheres these fibers were sometimes visible and could be seen with a faint neutral red stain. Various other muscle fibers were observed in living oncospheres. However, the continual movement made their representation impossible. An irregular network of contractile fibers was conspicuous beneath the cuticle. In this hexacanth embryo, fibers were more easily observed than in other oncospheres studied. A very heavy ring of short thick transverse bands appeared around the hooks (Fig. 9) in sectioned oncospheres. These were similar to bands described earlier for oncospheres of *Mesocestoides* and *Oochoristica*.

## DISCUSSION

Many cytological problems are involved in the development of the hexacanth embryo. Exactly how the various myoblasts produce contractile fibers and bring about movements of the oncosphere remains to be learned. What are the conditions that give rise to the rhythmic contractions of oncospheres? The identification of cortical and medullary contractile systems has been introduced to supplement the protractor and restractor systems of earlier writers. The latter terms refer more specifically to fibers attached to the hooks than to the distribution of functional musculature in the body of the oncosphere. The writer suggests that the cortical system in oncospheres is homologous with the subcuticular system of the metacestode and represents largely circular and constricting fibers, along with myoblasts that extend and pull the lateral hooks posteriorly. The antagonistic medullary contractile system is homologous with the longitudinal muscle fibers of the metacestode, and serves to retract the hooks and shorten the oncosphere. The medullary system develops from specialized embryonic cells forming a contractile center. In the hymenolepid-type embryo, this primordium is binucleate.

Where is the medullary contractile center or its homologue in oncospheres of the taeniid or mesocestoidid types? Restudy of the writer's slides showed that plastin cells stained prominently in the region behind the hooks were associated with a complex system of fibers. With proper stains these relations were demonstrated. The relation of plastin or germinative cells with compact stained cytoplasm, to a contractile system has been indicated (Ogren, 1956: p. 421). In early oncospheres of *Mesocestoides* the plastin cells were grouped in binucleate clusters. Moreover, in the metacestode and adult of *Mesocestoides*, longitudinal muscular systems developed early from the germinative type of cell and may set up a pattern for the proglottid. In describing oncosphere movements one must consider not only the type of embryo being used, but the cytology of cortical and medullary systems. Considerable comparative cytological and descriptive work remains to be done.

The hook is one of the most interesting differentiations in the tapeworm embryo. How oncoblasts can produce hooks of characteristic shape has not been revealed. Their extremely small size makes observation difficult. Somehow the granules of

the oncoblast are related to the production of the hook, perhaps in a secretory function. However, we have very little knowledge of what actually is taking place here from a biochemical standpoint. The medial hooks tend to be more slender, with, in some cases, a longer shank and more pointed blade. Is the longer shank related to heavy fibers attached to the medial extremity and to their constant pull against the hooks? Are the collar and barbs caused by the attachment of the oncoblast, or by the attachment to the cuticle and cortical muscles? The muscle fibers (Fig. 7) exerting a continual pull against the collar could conceivably produce the barbs. How is the curved blade produced? It certainly begins as a narrow cone. Later it broadens and becomes curved as it elongates in the cell. It is curved when still enclosed by a delicate membrane. Is it bent as it pushes against the cell membrane? The chemical nature of the hook is incompletely known. Is it a protein gel? Is it scleroprotein of the keratin type as suggested for rostellar hooks by Crusz (1947)? Are the blade and shank formed from the same cell, from separate cells, or in some cases from separate cell groups as suggested by the work of Clapham (1942) for rostellar hooks?

We are not yet certain of the taxonomic importance of hook morphology. The use of hooks to reveal phylogenetic relationships has been suggested by Kates and McIntosh (1950). They also suggested the use of hooks of the embryo to identify the metacestode of certain anoplocephalid worms. The correlation of hook morphology with the type of oncosphere, employed by Ogren (1957), may also lead to the recognition of phylogenetic relationships. Variations in hook morphology were summarized by Hwang and Kates (1956). These reports indicate that further work on hooks of oncospheres is desirable. However, the morphology and development of the hook should not be separated from the type of oncosphere that produces it. Moreover, it is necessary to describe carefully each pair of hooks since they may differ in morphology.

## SUMMARY

The morula, developed as the result of cleavage, became an oncosphere by developing cuticle, hooks, muscular parenchyma and epidermal glands. The morphology presented in this paper deals with structure and development of hooks, appearance and arrangement of embryonic muscle. Remarks are made concerning a cortical contractile system and a medullary contractile system. It is suggested that these are homologous with the circular and longitudinal muscle systems of the metacestode.

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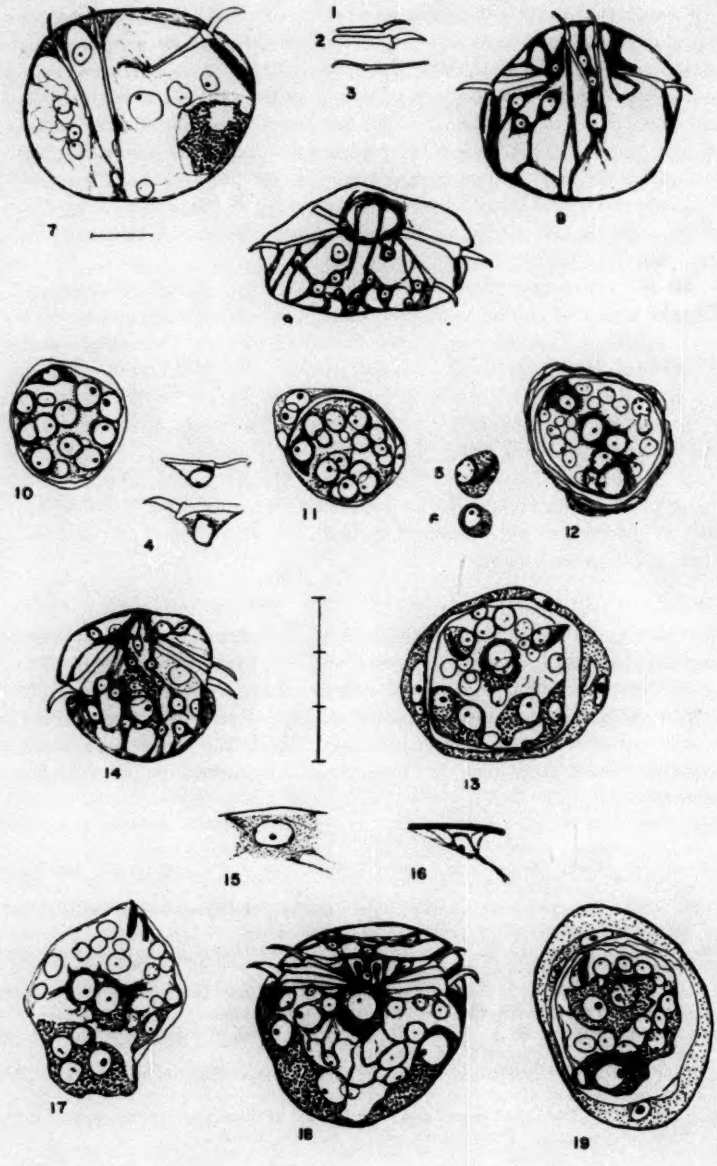

EXPLANATION OF FIGURES

All Figures made with the aid of camera lucida attachment. Each scale division represents 0.01 mm. Oil immersion objectives, 95×, 97×, were used for all studied. Figures 15, 16 are free hand. Figure 7 has its own scale. Abbreviations used: toluidine blue (TB), Lillie's metachromatic stain with acid hematoxylin and safranin O (MHS).

1. Dorsal-lateral hook.
2. Ventral-lateral hook.
3. Medial hook.
4. Lateral hooks showing oncoblast.
5. Oncoblast with cone-shaped blade, MHS.
6. Oncoblast with blade, or shank, shown as black rod cut in cross section, MHS.
7. Ventral view of oncosphere showing medial hooks with barbs and muscle attachments to cuticle. Lateral hook shows attachment of fibers to both sides of collar, aniline acid fuchsin stain.
8. Oncosphere showing some of myoblast associations, aniline acid fuchsin procedure.
9. Oncosphere to show associations in cortical parenchyma and muscular ring, aniline acid fuchsin procedure.
10. Early morula, TB, enclosed by thin shell.
11. Early morula with dark stained oncoblast, and nuclei of secondary macromere outside of morula, morula enclosed by shell, TB.
12. Morula with 3 primordia. At top left the darker cell is an oncoblast. In the middle the 2 dark nuclei are the binucleate primordium. At the bottom the dark granular cells are the forerunners of the epidermal glands, oncosphere is enclosed by shell capsule and some membranes of pseudoembryophore, TB.
13. Early oncosphere enclosed by shell and developing pseudoembryophore. Hook blades are forming in the oncoblasts. The binucleate primordium, in the middle, will form the medullary contractile center. The epidermal glands at the bottom are filled with granules that stained red with safranin O, MHS.
14. Oncosphere, more advanced than Figure 13, designed to show the fiber relations of the medullary contractile center in the middle and other fibers of parenchyma visible in this section. The cut ends of the medial hooks, shown as 2 dark circles, are located just above and medial to the lateral hook shanks, stained with aniline acid fuchsin.
15. Early cortical parenchyma cell, free hand and diagrammatic, from oncospheres similar to Figures 11, 12. The thin cuticle is shown by the upper dark line, MHS.
17. Early oncosphere, lateral view, to show the 3 primordia in a later stage of development, hooks are present at top, muscle fibers pres[illegible] from the contractile center in middle, and epidermal gland below is prominent, MHS.
16. Cortical parenchyma cell, free hand and diagrammatic, from an oncosphere similar to Figures 9, 14, MHS.
18. Mature oncosphere, aniline acid fuchsin procedure.
19. Early oncosphere, enclosed by shell and the developing pseudoembryophore, MHS.

## RESEARCH NOTE

### *THELAZIA CALIFORNIENSIS* FROM A MULE DEER, *ODOCOILEUS HEMIONUS CROOKI* (MEARNS, 1879), IN NEW MEXICO

On November 16, 1957, 4 female eyeworms were collected by one of us (R.W.R.) from a mule deer, *Odocoileus hemionus crooki* (Mearns, 1897), in the Gila Wilderness Area, Grant County, New Mexico. These were found to fit the redescription of *Thelazia californiensis* Price given by Kofoid et al (1937, U. Calif. Publications Zool. **41**: 225–233).

According to the recent survey of Parmelee et al (1956, J. Am. Vet. Med. Assoc. **129**: 325–327), *T. californiensis* has been reported to date only from Oregon, California, and adjacent Nevada; several of these reports cite deer as hosts. The authors are not aware of any published record of *T. californiensis* except from the Pacific coast and adjacent area and therefore desire to call attention to the presence of the nematode in New Mexico.

This work was done in cooperation with the New Mexico Agricultural Experiment Station.—G. A. SCHAD, *Animal Disease and Parasite Research Division, ARS, U.S.D.A., State College, New Mexico,* AND R. W. RAUGHT, *New Mexico Department of Game and Fish.*

# STUDIES ON THE DEVELOPMENT OF *DAVAINEA PROGLOTTINA* IN THE INTERMEDIATE HOST

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Information on the development and life cycles of tapeworms has accumulated more slowly than that relating to morphology. Few tapeworm life cycles are completely known. In *Davainea proglottina,* frequently considered to be a pathogen of poultry, the life history involves a slug as the intermediate host. The role of these molluscs was demonstrated by Grassi and Rovelli (1888), Chandler (1923), and Wetzel (1932). These authors showed that the following may serve as intermediate hosts: *Agriolimax agrestis, Limax cinereus, L. flavus, Arion empericorum* (= *A. ater?*) *A. hortensis, A. circumscriptus,* and *A. intermedius.*

The present author has confirmed these observations and has also been able to infect the slugs, *Agriolimax reticulatus, A. caruanne, Milax gracilis,* and *M. sowerbyi.*

Since the interruption of life cycles has frequently proven to be effective and relatively inexpensive in controlling parasitic infections, the life cycle of *Davainea* has been studied with the aim of eventually developing methods of controlling this infection of poultry.

## MATERIALS AND METHODS

Slugs were collected from their habitats in moist, shady places, such as under stones, wood or on leaves of cabbage, lettuce, and other plants. The slugs were frequently found to be buried in the soil. These soft-bodied animals varied considerably in size. To preclude natural infections, slugs were collected from farms, gardens and parks to which poultry had no access. In the laboratory, slugs were maintained in glass vessels 2×3 feet in width and length and 2 feet high. They were covered with a piece of turf on which stones were placed. The turf was sprinkled with enough water daily to keep it slightly moist. The slugs bred in these containers. When about a month old, young slugs were removed to other containers and were available for experimental work. *Agriolimax reticulatus, Arion ater A. hortensis,* and *A. circumscriptus* were successfully bred under these conditions.

Several fowl harboring *D. proglottina* were obtained. One cock passed hundreds of segments daily and served as a constant source of infective material for more than a year. The infected bird was kept in a wire-bottomed cage over a pan of water. Motile segments were recovered from freshly passed feces. Slugs, previously fasted for 24 hours, were placed in test tubes lined with moist paper and fed gravid proglottids on small pieces of lettuce. Each slug was kept in the tube overnight. In some experiments, the slugs were fed gravid segments under observation with a low power microscope. This was done to determine how many segments were eaten and when. Infected molluscs were maintained as indicated above and fed on clean lettuce, cabbage, and potatoes. The temperature of the laboratory was about 21°C. When experimental slugs died they were dissected for study of larval tapeworms. Animals were also sacrificed at intervals to study the details of worm development. All such material was fixed in 10% formol-saline. Whole mounts were stained with acetic acid—alum carmine and studied in glycerine jelly. Serially sectioned material was stained with haematoxylin and eosin and mounted in balsam.

## DEVELOPMENT OF THE CYSTICERCOID

The onchosphere is liberated in the gut of the slug. The mechanism of liberation is not known. The free onchosphere is a greyish, granular mass, ovoid or irregular in shape, surrounded by a delicate membrane. The tips of the 3 pairs of

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onchosphere hooks project through this membrane during movement. During movement, the middle pair of hooks swing up and down while the pairs on either side move in a plane at right angles to that of the middle pair. The pairs on either side are moved forward and outward, resembling the breast stroke of a swimmer. After 1 to 2 hours in the slug the onchosphere attaches itself, apparently by means of the middle pair of hooks, to the wall of the gut (Fig. 1). In sections a slug fixed an hour after it had ingested onchospheres, the point of penetration in the gut wall could be seen clearly. Twenty-four hours after ingestion, the onchospheres had penetrated the gut wall, some of them being found in the body cavity between the stomach and the salivary gland and some between the intestine and the digestive gland (Fig. 2).

The larval cestodes increased considerably in size during the week after infecting the slugs and could then be detected as light spots on the outside of the gut. Small spaces within the larva coalesced through the degeneration of internal cells, forming a single cavity (the primitive lacuna). During this period, a fibrous layer, perhaps of host origin, formed around the larva (Fig. 3). One end of the larva underwent differentiation and at 12 days the rostellum and suckers were distinguishable (Fig. 4). Apparently, the scolex was then withdrawn into the cavity of the larva, resulting in a double-walled cyst. The space between these 2 walls is the primitive lacuna, which was subsequently filled by parenchymatous tissue through proliferation of cells from the wall. Shortly afterward, numerous calcareous bodies were formed.

The mature cysticercoids were detectable as white spots in contrast to the pink color of the mollusc's gut. They were usually oval in shape, but this varied somewhat, especially in heavy infections. In size, the cysticercoids varied from $250 \times 190$ to $150 \times 120$ microns. The cysticercoid has a rough outer coat, described by Chandler (1923) as a gelatinous, wrinkled coat. In the living cysticercoid (Fig. 8), a thin, light-refracting cuticle was observed. Internal to the cuticle is a granular subcuticle, variable in thickness but with greatest width on the side opposite the invagination canal. Study of sectioned material showed that this zone is composed of numerous large, elongated cells and is separated from the finely striated cuticle by a basal membrane (Fig. 10). A thin fibrous layer delimits the cardioid inner body of the cysticercoid. Just beneath this fibrous layer and surrounding the scolex, are numerous calcareous bodies, uneven in shape and 8 to 10 microns in diameter. The details of structure of the scolex are difficult to distinguish, owing to the presence of the calcareous bodies. This difficulty was obviated by inducing excystment. In normal saline at 40° C contraction of the cyst wall and movement of scolices were initiated, and the scolices emerged from the opening through which invagination had occurred (Fig. 10). Addition of artificial gastric juice (0.5% pepsin in 0.2% HCl) aided the process of excystment, apparently digesting the outer covering of the larva. The scolex is armed with 2 rows of rostellar hooks. The suckers are armed with several rows of hammer-shaped hooks. It was profitable to count the rostellar hooks on the excysted larvae since they are readily lost from the mature worm; they varied from 64 to 84 in number. The development of a well defined caudal appendage in the cysticercoids could not be detected. Grassi and Rovelli (1892) showed a young cysticercoid of *D. proglottina* with a short caudal appendage bearing 1 pair of hooks which was also noted by Braun (1894). Wetzel (1932)

stated that in the many cysteroids studied by him only 1 with such a stump-shaped appendage was found. During this work no cysticercoids with such an appendage were noticed, but many of them had what appeared to be a rudimentary caudal appendage (Fig. 5). Sometimes, but not always, at 1 end of the cysticercoid, a part of the body bearing a pair of hooks becomes separated off by a septum (Fig. 6). The separated portion has usually a sickle- or crescent-shape. Such cysticercoids have been observed by Kalkus (1928) and Wetzel (1932). These cysticercoids appear as globular, milky-white bodies on the outer wall of the alimentary tract of the slug, especially in its anterior third (stomach) (Fig. 7). The cysticercoid takes about 3 weeks to reach maturity, much depending on the temperature of the environment in which slugs are kept. In this experiment it reached maturity in 18 days when the laboratory temperature varied around 21° C. In heavily infected slugs cysticercoids were found almost throughout the contents of the body cavity. In several specimens (*Agr. reticulatus*) up to 50 mature cysticercoids were found in each slug, their presence not affecting these slugs in any apparent way.

### EFFECT OF TEMPERATURE ON DEVELOPMENT

Forty laboratory-reared *A. reticulatus* were given freshly collected segments of *D. proglottina*, as described above. Thirty-four of them fed on the worm material and were separated in 2 equal groups (I and II) and kept in Petri dishes lined with moist paper. Group I was kept in an open field under a tree; the temperature varied around 15° C. The animals were fed clean lettuce and the filter paper changed every other day. Ten days after exposure 1 slug died and on dissection numerous developing cysticercoids were found. Two more died on the 12th day and were found to be harboring immature tapeworm larvae. Every second day from the 18th to the 28th day, 2 slugs were killed. In each case recovered cysticercoids were fed to a clean 3½-month-old, cage-reared fowl. Two weeks after receiving cysticercoids, the droppings of each fowl were examined for gravid segments. This was repeated daily for 1 week. Segments were recovered only from birds fed 26- and 28-day-old cysticercoids.

The slugs of Group II were kept in similar dishes in an incubator at 25° C. Mortality was high, 2 slugs dying on the 6th day, 1 on the 7th, and 5 on the 8th day. Six of these slugs harbored developing cysticercoids. On the 11th day 3 slugs showed cysticercoids in an advanced stage of development. Two slugs were killed on each alternate day from the 11th through the 15th day after infection. As with Group I, recovered cysticercoids were fed to clean chickens. Each of the 3 chickens was examined as indicated above, and segments were recovered from birds fed 13 and 15 day old cysticercoids.

Thus, at a temperature of about 15° C the cysticercoids required 26 days to attain a state infective for fowl. At 25° C, the infective state was reached in 13 days. Environmental temperature is obviously a factor determining the rate of development of *D. proglottina* in the intermediate host.

### SUMMARY

1. Eleven species of slugs were shown to be capable of acting as the intermediate hosts of the fowl tapeworm, *Davainea proglottina*.

2. Four series of slugs were bred under laboratory conditions.

3. The development of the cysticercoid of *D. proglottina* was studied. The

hexacanth embryo hatches in the gut of the slug and penetrates the gut wall. The larva grows rapidly with the formation of an interior space, the primitive lacuna. One pole of the cyst differentiates to form the scolex which is withdrawn into the cavity of the cyst before maturation. A rudimentary caudal appendage was observed on the cysticercoids.

4. The rate of development of cysticercoids is dependent on the environmental temperature of the slug.

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## Explanation of Figures

Figure 1. Section through the intestinal wall of *Agriolimax reticulatus* showing the penetrating onchosphere 1 hour after feeding the host on proglottids of *D. proglottina*.

Figure 2. Section through the intestine of *A. reticulatus* 24 hours after infection.

Figure 3. Section through on early larva of *D. proglottina* (7 days) shownig the outer fibrous coat (f.c.) and the primitive lacuna (p.c.).

Figure 4. Section through a 13-day cysticercoid before withdrawal of the scolex (s).

Figure 5. Several mature cysteroids showing the caudal appendage (c.a.).

Figure 6. Section through a cysticercoid in the process of scolex-withdrawal (15 days). Caudal appendage (c.a.) may be seen.

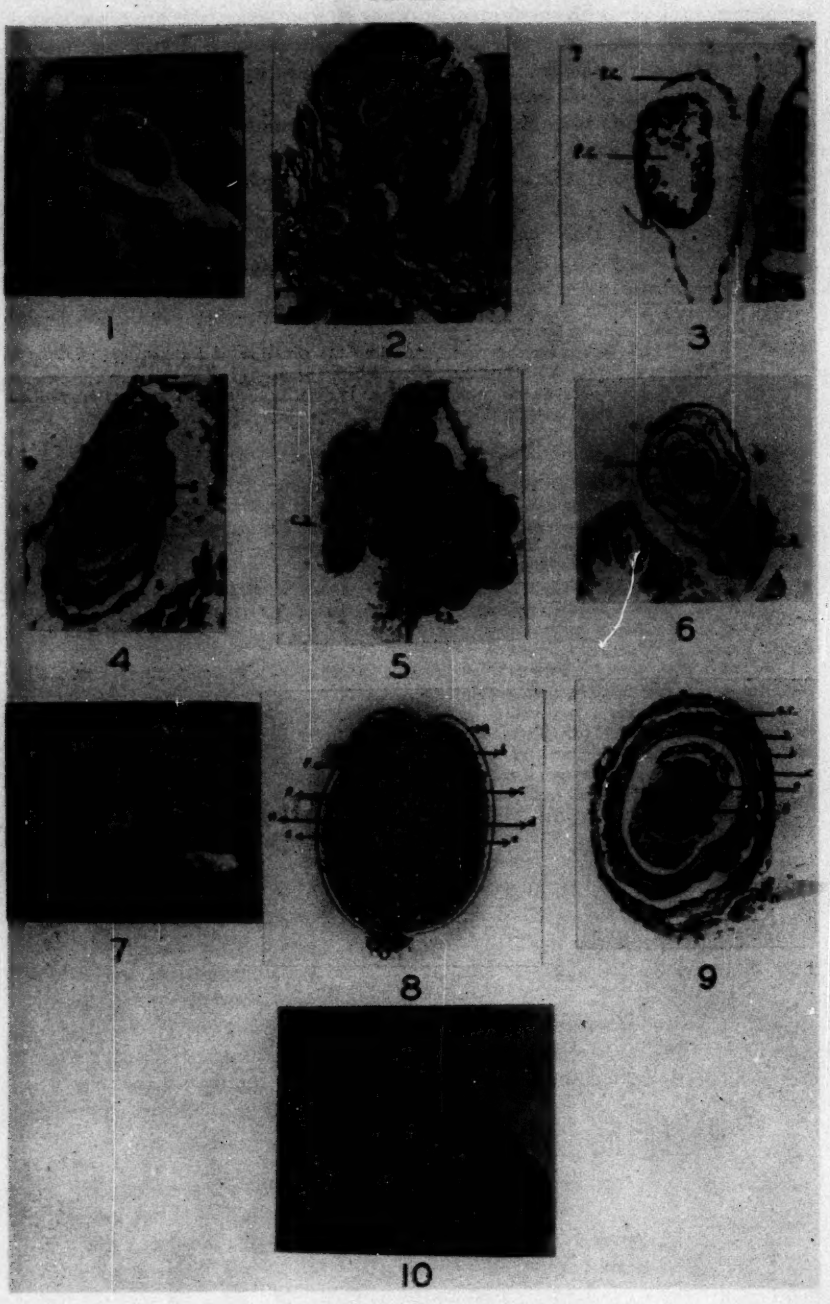
Figure 7. Body cavity of infected *A. reticulatus* opened by incision of the lateral line. White spots are mature cysticercoids of *D. proglottina*.

Figure 8. A living systicercoid. Distinguishable in the photograph are cuticle (a), basal membrane (b), subcuticle (c), fibrous layer (d), inner cyst wall (e), calcareous corpuscles (f), rostellum (g), suckers (h), and the scolex (s).

Figure 9. Section through a mature cysticercoid showing some of the structures seen in Figure 8, in addition to the outer fibrous coat (a.c.).

Figure 10. An excysted cysticercoid. Abbreviations as in Figures 8 and 9.

PLATE I



# SEASONAL VARIATIONS IN *BAERIETTA DIANA*

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Seasonal cycles in cestodes have rarely been studied, Oliger (1950) and Sudarikov (1952) having provided virtually the only reports in the literature. The present study is based on the collection record of *Baerietta diana* (Helfer 1948) Douglas 1958 which occurs in *Batrachoseps attenuatus* on the west coast.

Although the collections upon which this report is based were not made with a view to statistical rigor, the data are given here in the hope that more extensive studies will be made. All the host salamanders were obtained in the Berkeley Hills near the campus of the University of California. They were not collected at regularly spaced intervals nor were they collected at definte stations. All hosts were decapitated; the worms were transferred to chlorotonized Ringer's solution and measured in a relaxed (living) condition.

## OBSERVATIONS AND DISCUSSION

The most revealing data are those concerning the incidence of infection (see Fig. 1). Salamanders collected in September and October were invariably unin-

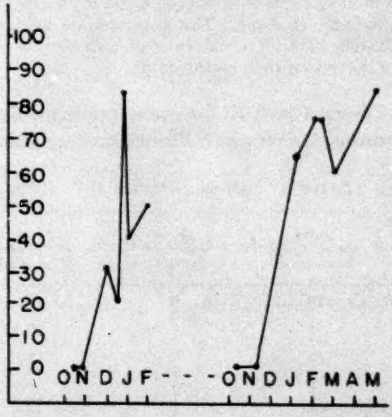


FIGURE 1. A plot of the incidence of infection. The ordinate indicates the percentage of host salamanders infected; and the abscissa is a time scale beginning October, 1950, and ending May, 1952.

fected even though they included adults as well as immature specimens. Infected salamanders were not found until November, but the percentage of infected hosts increased from that time until May. During the dry months (June–September) salamanders are virtually unobtainable so that data are not available for that period. Even so, the simplest explanation for the observed annual fluctuation is that sala-

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manders become defaunated during the summer months and are subsequently reinfected in the fall, winter, and spring.

Examination of the length data (see Fig. 2) supports the above hypothesis.

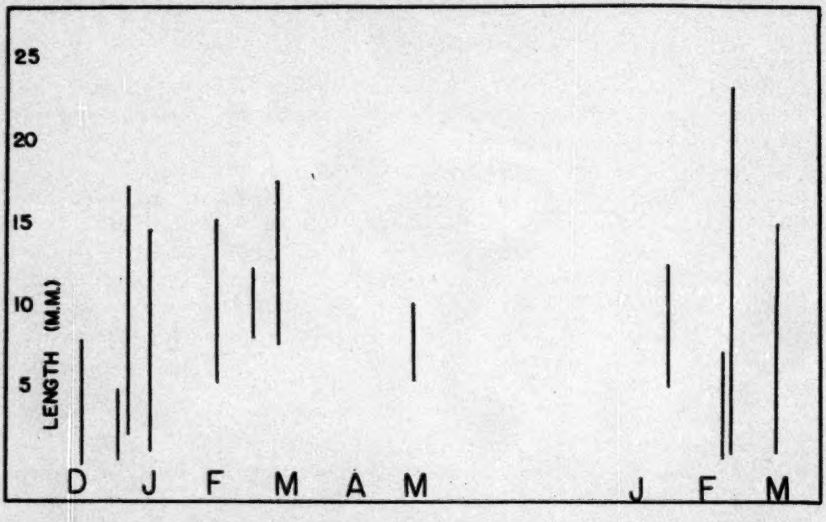


FIGURE 2. Length data for *B. diana*. The time scale on the abscissa begins with December, 1950, and ends in March, 1952. The extremes of each plotted line represent the minimum and maximum lengths of worms on each collection day.

The average length of worms early in the cycle is much less than later in the winter and spring. Furthermore, the standard deviations (see Table I and Fig. 2) imply

TABLE I. *Data on collection of* B. diana.

| Date | Total no. of hosts examined | no. of infected hosts | Mean length (mm) | Mean no. of worms per host† |
|---|---|---|---|---|
| 10/12/50** | 10 | 0 | —— | — |
| 10/23/50** | 7 | 0 | —— | — |
| 11/30/50 | 3 | 1 | 2.1 ± 2.1* | 10.0 |
| 12/16/50 | 5 | 1 | 1.8 ± 1.04 | 7.0 |
| 12/20/50 | 11 | 9 | 11.0 ± 4.7 | 5.3 |
| 12/30/50 | 5 | 3 | 8.0 ± 5.1 | 4.0 |
| 1/30/51 | 2 | 1 | 8.8 ± 2.9 | 8.0 |
| 2/21/51 | 1 | 1 | 10.0 ± 2.0 | 3.0 |
| 2/24/51 | 1 | 1 | 10.8 ± 3.4 | 6.0 |
| 4/22/51 | 1 | 1 | 7.3 ± 1.5 | 6.0 |
| 10/10/51 | 5 | 0 | —— | — |
| 11/ 8/51 | 3 | 0 | —— | — |
| 1/16/52 | 3 | 2 | 4.5** | 8.0** |
| 2/ 6/52 | 5 | 4 | 4.3 ± 1.3 | 7.0 |
| 2/12/52 | 6 | 5 | 10.2 ± 5.5 | 5.2 |
| 3/ 1/52 | 10 | 7 | 7.5 ± 3.3 | 4.6 |

* Standard deviation.
** Approximate.
† Not including uninfected hosts

the existence of only 1 worm age-group in November; while later in the year, the wider variation in worm length suggests that worms of different age groups exist in nearly every sample. Although it is possible that worms of a single age group could vary so extremely in size, it does not seem likely. The smaller worms taken later

in the spring are undeveloped and are without reproductive organs. Also they have an "excretory bladder" which is lost in older forms.

Figure 3 demonstrates that the number of worms per host decreases later in the

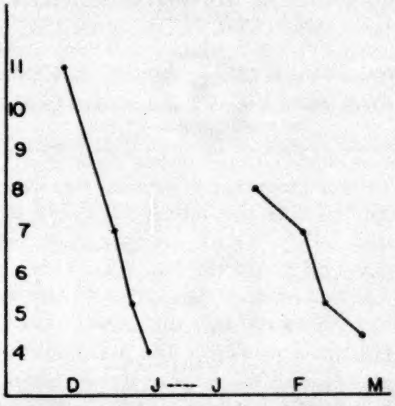


FIGURE 3. The average number of worms per host salamander. The ordinate represents the average number of worms per infection. The time scale on the abscissa covers the period: December, 1950–March, 1952.

cycle. This implies that some of the smaller worms observed earlier in November and December do not become permanently established in the gut.

## SUMMARY

Examination of the collection record of *Baerietta diana* (Helfer 1948) from *Batrachoseps attenuatus* suggests that infection occurs late in the fall and that after about 8 months of growth, the worms die.

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# PARASITEMIA AND SURVIVAL IN MICE INFECTED WITH *PLASMODIUM BERGHEI*. HYBRIDS BEWEEN SWISS (HIGH PARASITEMIA) AND STR (LOW PARASITEMIA) MICE

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In the mouse the percentage of mature erythrocytes infected with *Plasmodium berghei* at the end of the first week post-inoculation varied with the strain of mouse (Greenberg and Kendrick, 1957). Of the strains studied, Swiss mice had the highest parasitemia (47%) and STR mice had the lowest (5%). In these experiments Swiss mice had been used as common donors for all the strains of mice. There was a possibility that the abnormally high parasitemia observed in Swiss mice was a reflection of a Swiss-adapted parasite. The hypothesis was tested (Greenberg and Kendrick, 1957a) by passing the parasite serially through each of the strains of mice. There was no evidence that in any of these strains of mice there was an increase in parasitemia with continued serial passage. The high parasitemia in Swiss mice was then not entirely a matter of parasite-adaptation.

It was thought that the degree of infection of mature erythrocytes achieved in each strain was genetically controlled. Something could be learned of the mechanism of inheritance of resistance to parasitemia by studying hybrids and backcrosses of the 2 extreme examples, Swiss and STR. It was expected that segregation and recombination of genes could most easily be determined with strains showing the largest differences.

## METHODS

All mice except the parent STR/N and Swiss were bred in this laboratory. The Swiss and STR mice were kindly furnished by Dr. George E. Jay, Jr. of the National Institutes of Health.

Fifteen mating pairs of STR and Swiss were set up. Eight of these were Swiss female and STR male and the rest were the reciprocal. The first litter of 1 of these families was used for producing the F2, backcross to Swiss and backross to STR mice. This family had no subsequent litters. The offspring of 1 other family were used to produce backcross mice. The number of families, litters, and the sex of the offspring of each mating, which survived long enough to be used in the experiments, are shown in Table I.

TABLE I. *Distribution of experimental mice by strain, family, litter, and sex.*

| *Strain* | *Families* | *Litters* | *Males* | *Females* |
|---|---|---|---|---|
| Swiss | | | | 344 |
| STR | | | | 98 |
| Swiss × STR $F_1$ (and reciprocal) | 14 | 47 | 162 | 176 |
| Swiss × STR $F_2$ | 3 | 21 | 86 | 62 |
| (Swiss × STR) × STR | 4 | 29 | 110 | 101 |
| (Swiss × STR) × Swiss | 6 | 28 | 87 | 85 |

The mice were inoculated when 2 months old. Each mouse received through a tail vein 1 million parasitized erythrocytes from a Swiss donor . At each inoculation 5 to 10 Swiss mice were also inoculated and served as controls. During the period of the present experiments 4 groups of female STR mice were also inoculated. The remainder of the STR groups were inoculated earlier.

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* Laboratory of Tropical Diseases.

Blood smears (tail blood) were made on day 6 and occasionally on days 5 and 7. These were stained with Giemsa and read for the percentage of mature erythrocytes infected. The survival of each mouse was noted.

## RESULTS

A preliminary analysis of the data showed that the variance of the data increased as the mean increased. This was largely rectified by performing a square root transformation. Within families there was a significant sex difference, males having a significantly lower count than females. When this analysis was made within strains and by litters (Table II), in every comparison the males had lower infections and

TABLE II. *Mean sixth-day parasitemia of mature erythrocytes by litter or inoculation group and by sex in mice infected with* P. berghei.

| *Strain* | Mean percentage 6th day parasitemia (mature erythrocytes) | |
|---|---|---|
| | *Males* | *Females* |
| Swiss | | 48.40 ± 0.9 (48) |
| STR | | 5.0 ± 0.4 (13) |
| Swiss × STR $F_1$ | 12.34 ± 1.2 (46) | 15.94 ± 1.4 (46) |
| Swiss × STR $F_2$ | 8.14 ± 0.9 (20) | 16.05 ± 2.2 (20) |
| (Swiss × STR) × Swiss | 13.78 ± 1.6 (29) | 23.38 ± 2.1 (29) |
| (Swiss × STR) × STR | 7.11 ± 0.8 (27) | 12.70 ± 1.9 (27) |

(Figures in parenthesis are litters.)

in all but 1 strain the difference between sexes was significant. Within families and strains there was a significant difference between litters. This was probably of the same nature as was earlier observed between groups of a single strain inoculated at different times (Greenberg and Kendrick, 1956). No further significance can be attributed to this observation than that when otherwise comparable mice are inoculated at different times, differences between the groups larger than can be expected of animal variance will be observed. Finally, with the exception of backcross to Swiss mice, there were no significant differences among the families when the comparisons were made by litter means. One of the families of backcross to Swiss appeared to have a somewhat higher parasitemia than other families in the group. While this may be biologically significant, it may be best to postpone any decisions about its significance until confirmatory evidence can be acquired. On the basis of the present evidence we can assume that the difference was due to chance.

Comparison among the mating groups has been done by sex and by litter. For instance the arithmetic mean of parasitemia of all the females within a litter was calculated. Then the mean of all the litters was averaged and the estimate of confidence limits based on this calculation. This procedure is perhaps wasteful, but it is conservative and takes cognizance of the variation between litters or inoculation groups.

Female Swiss mice had counts significantly higher than all other mice ($p < 0.001$); female STR had counts which were significantly lower than all other groups of mice ($p < 0.001$). The backcross to Swiss mice had counts significantly higher than backcross to STR mice ($p < 0.001$), but the difference was not significant among all other hybrid groups of mice.

The male backcross to Swiss mice had counts significantly higher than the backcross to STR mice but among the other possible comparisons there were differences which were suggestive but not significant. It is noteworthy that among both males and females the backcross to Swiss mice had the highest counts of the experimental

groups and the backcross to STR the lowest, but the relation between F1 and F2 mice was different for males and females.

The survival of the mice is given in Figures 1 and 2 and Table III. The survival of F1 and F2, and backcross to STR was remarkably similar in terms of percentage of mice surviving day 10, day by which 50% were dead, average survival, and general pattern of survival. (The mean data are given as an overall expression with no implication that the distribution of deaths is normal.) These 3 groups of mice lived longer than either parent. So, in fact, did the backcross to Swiss mice though the difference between them and STR mice was small.

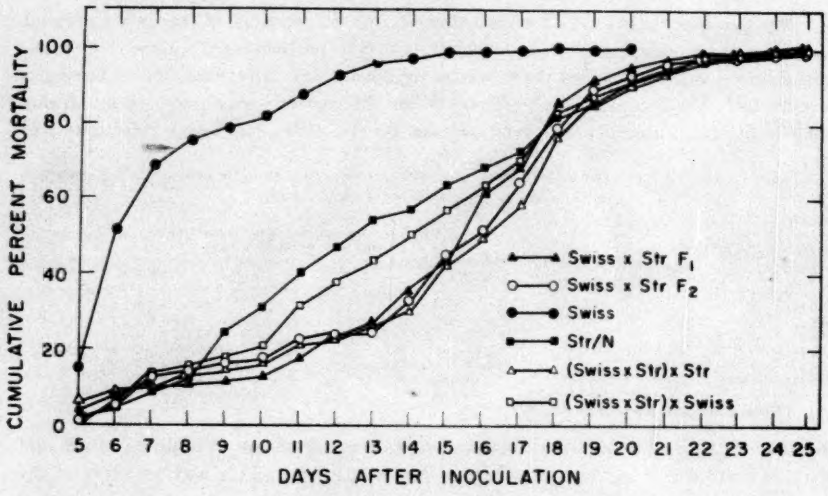


FIGURE 1. Cumulative per cent mortality in several strains of mice.

## DISCUSSION

It is apparent that there is a distinct sex difference in each of the groups studied, with males having, as a whole, lower infections than females. In all the groups but the F1, the ratio of counts male/female was between 0.5 and 0.6. This agrees with earlier observations (Greenberg and Kendrick, 1957) in C57 B1, A, and BALB mice. The F1 male/female ratio of 0.77 is then unusually high. While it is possible that there may be sex-strain interactions it seems more likely that the estimate for male F1 mice was high or that for females was low. Unfortunately there is no sure way of deciding which without gathering more data. It would seem more reasonable to believe that the male estimate was high, which would imply that there was essentially no difference in the parasitemia between F1 and F2 mice. All of this points out the difficulty of making decisions about differences in parasitemia where these differences are small and the variability of the data large.

Regardless of which way the male-female impasse in F1 is decided, the hybrids had significantly lower infections than their Swiss parents and higher counts than their STR parents. This could be interpreted to mean that the determinants of low parasitemia are at least partially dominant over those for high parasitemia. How-

ever, taking the data as they stand, the average parasitemia of F2 mice was no higher than that of F1 mice. There was then no evidence of segregation and recombination of alleles of a single gene. One would expect that were a single gene involved, ¼ of the mice would resemble STR mice, ¼ Swiss, and ½ F1 hybrids. Such a combination would have an estimated count of about 21% which is more than 2 standard errors beyond the observed female mean. The backcross to Swiss would be expected to have counts of about 32% and this is more than four standard errors beyond the observed. Backcross to STR would be estimated at 10.5% which is somewhat lower than that actually observed for females. The data obviously disagree with any hypothesis of a single gene effect.

TABLE III. *Survival in mice infected with* P. berghei.

| | % Dying in 10 days or less | 50% dead by day | Mean Survival (days) |
|---|---|---|---|
| Swiss | 81 | 6 | 7.7 |
| STR | 31 | 13 | 13.6 |
| Swiss × STR $F_1$ | 12 | 16 | 15.2 |
| Swiss × STR $F_2$ | 17 | 16 | 15.2 |
| (Swiss × STR) × STR | 16 | 16 | 15.4 |
| (Swiss × STR) × Swiss | 21 | 14 | 14.3 |

The data suggest that the malarial infection of the mature red cell in mice is controlled by more than 1 gene. The F1 hybrids were intermediate in their parasitemia between the parent strains, as were the F2 mice. Backcross to STR mice approached the STR parents and backcross to Swiss mice approached the Swiss parents in parasitemia. However, if many genes were involved, we should expect that the F2 data would have shown more variance than the F1, and for this there was no evidence. About the same percentage of F1 as F2 mice had counts of less than 10% (individuals, both sexes), 49% for the former as compared with 54% for the latter. In the range of 10 to 20% parasitemia, there were 29% of F1 individuals and 32% of F2 individuals. Eight percent of F1 mice had counts over 30% while 6% of F2 mice had parasitemia in this range. The distribution of data in these 2 groups was similar. On the other hand 68% of backcross to STR mice had counts of less than 10%, while only 32% of backcross to Swiss individuals had counts this low. Only 2% of the backcross to STR mice had counts over 30% while 18% of the backcross to Swiss had counts that high. The factors which control reproduction of the parasite in the mature red cell appear to be inherited but in a complicated manner.

Turning to survival, the authors have been impressed for some time by the craggy nature of the survival curves. It had been felt that a sufficiently large sampling of a given population should smooth the curve. In 1 artificial pool of a large number of mice from a variety of genetic backgrounds, a somewhat smooth bimodal curve was derived (Nadel, Greenberg, Jay and Coatney, 1955). However, in the present series, the survival curve of Swiss mice (Fig. 2), involving 344 animals, all of 1 sex, was not much smoother than that of STR mice in which there were only 98 animals. In almost every group of mice one gets the impression that there are 1, 2 or 3 major modes of deaths. But what of the minor modes? Are these a reflection of real biological events or are they randomly distributed by chance? In previous series, we had been impressed with the fact that certain days, 6, 15, 18 etc., occur rather frequently as major modes among different strains of mice.

If all the modes, major and minor, are listed for each of the groups of mice in Figure 2, as is done in Table IV, there are 5 to 8 modes in a period of 15 to 27 days.

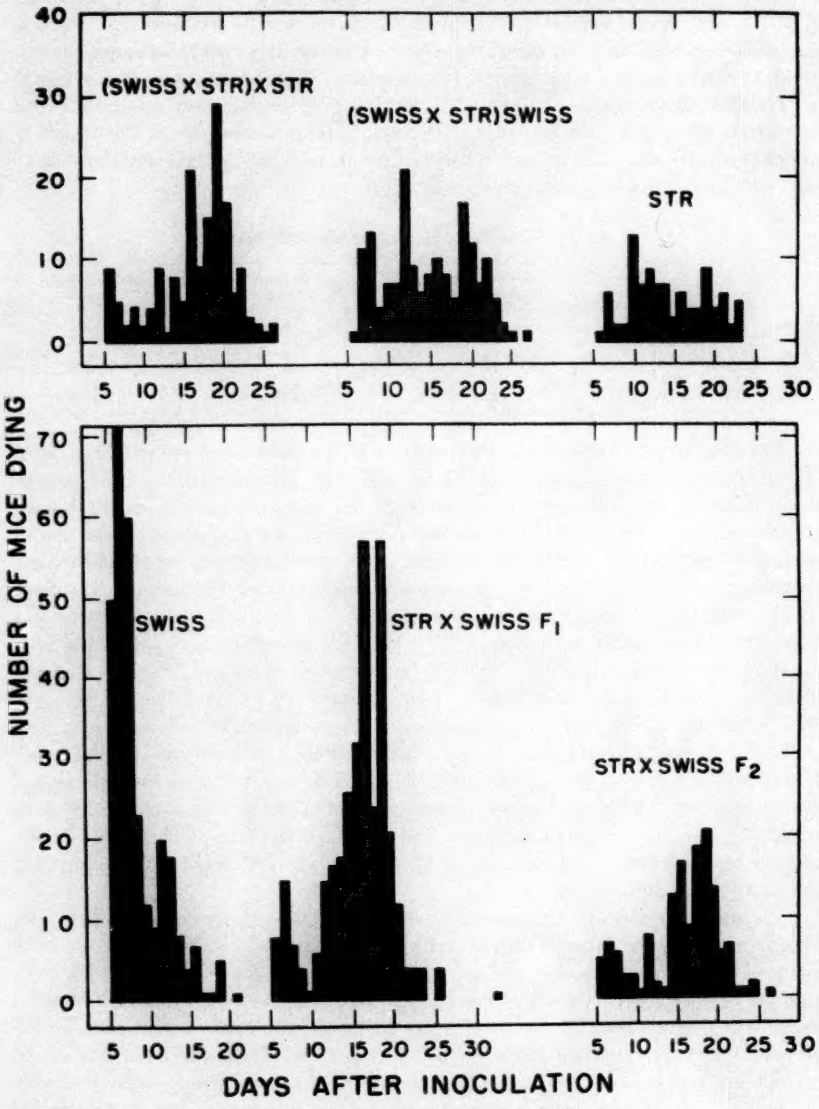


FIGURE 2. Mortality days after inoculation in several strains of mice.

The average number of modes per group was 6.3 and the average period of time 21 days, yielding an artificial mean interval of 3.3 days between modes. Certain days, or 2-day periods, occur with unusual frequency. All of the groups had modes in the

5- to 7-days period, the 15th day and the 18th day. Five are represented on the 11th day and the 20th or 21st day; and in all groups with any survivors there was a mode in the 24- to 26-day period.

TABLE IV. *Days on which major or minor modes of death occurred in several strains of mice.*

| *Strain* | *Days on which modes of death occurred* |
|---|---|
| Swiss | *6*, 11, 15, 18, 21 |
| STR | 6, 9, 11, 15, 18, 20, 22 |
| Swiss × STR $F_1$ (and reciprocal) | 6, *16*, 18, 25, 33 |
| Swiss × STR $F_2$ | 6, 11, 15, *18*, 21, 24, 26 |
| (Swiss × STR) × STR | 5, 8, 11, 15, *18*, 21, 25 |
| (Swiss × STR) × Swiss | 7, *11*, 18, 21, 26 |

Italic figures are major modes of death.

The mode of the 5- to 7-day period was the major mode of 1 group, the third highest mode of three groups. The mode on the 11th day was the first, second, or third highest of each group represented. That of the 18th day was the highest mode in 3 groups, second highest in 2. There appears then to be a regular succession of modes at about the 6th, 11th, 15th, 18th, 21st, and 25th days. The intervals appear to decrease as the infection progresses. Thus, from inoculation to first mode was 6 days; first to second, 5 days; second to third, 4 days; and thereafter there were about 3 days between modes.

While it is true that the present series represents a selected group of mice, this should not detract from the comparison. The fact that these mice were all rather closely related genetically should only emphasize the reproducibility of the phenomenon. Furthermore, somewhat similar cyclical modes can be discerned in previous results with different inbred strains of mice (Greenberg and Kendrick, 1957 and 1957a).

All of this suggests a regular, cyclical phenomenon which appears to put a maximum stress on the mice. Plasmodia are notoriously periodic in their growth and multiplication and one would be tempted to conclude that the modes of death represent a periodicity in *P. berghei*. However, among Plasmodia, *P. berghei* is notably acyclical. Still there could be a periodicity superimposed on what appears to be an acyclical growth curve. On the other hand the periodicity may apply to the host. One might speculate that the defenses of the host become saturated at regular intervals or that recuperative powers increase and decrease regularly.

None of the above should be construed as a retraction of earlier statements that longevity in mice infected with *P. berghei* is in general determined by (a) the percentage of mice which survive the first wave of deaths and (b) when the second or subsequent major modes of deaths occur. The first wave of deaths appears to be caused by factors different from that causing some, at least, of the later waves of deaths. (Highman, Greenberg and Coatney, 1954 and Greenberg, 1955).

If the cyclical nature of the death curves is real, then the intervals between modes are suggestive. One would expect that, as the host had further exposure and more experience at handling the parasite, the intervals between attacks should increase. On the contrary, as the infection proceeds, the intervals between modes appear to decrease. This suggests a progressive exhaustion of the host's means of defense or recuperation.

The picture then is as follows: There is a major assault on the mice at the end of the first week, involving a severe hemolytic crisis, probably accompanied by toxemia

and shock. Some strains of mice are better able to withstand this initial assault than others. Following this, there occurs a cyclical series of attacks producing a series of modes of death times. Which of this series will kill the most mice is again characteristic of the strain of mouse. One would like to know the immunological and physiological mechanisms involved in this series of events, as well as the number of genes and alleles which control them. These are obviously very complicated problems in which a great deal of preliminary probing is required, even before fundamental questions can be asked or answered.

Concerning the genetics of the current series of hybridizations, F1 mice lived longer than either parent, an observation in keeping with others made earlier with other hybrids (Nadel et al, 1955). The difference between the F1 hybrids and the Swiss parent mice was chiefly in the percentage of mice which survived the first wave of deaths. The difference between F1 and STR mice was in the percentage of mice which survived the first and second wave of deaths. Heterosis was not lost in the F2 or in the backcross to STR, but was less obvious in the backcross to the short-lived Swiss mice. Some useful information as to the number of genes involved may be obtained by observing how many generations of backcrossing or hybridization one would have to breed before the survival curves of the mice approximated that of either or both parents.

## SUMMARY

Swiss × STR/N F1 and F2 hybrids, as well as the F1 backcross to Swiss and STR, were inoculated with *P. berghei*. The 6th day parasitemia of mature erythrocytes was observed as well as survival.

The F1 and F2 hybrids had parasitemias significantly lower than that of the parent Swiss mice, but somewhat higher than that of the STR parent. There was no significant difference between the results in the F1 and the F2 mice. The backcross to STR mice had counts significantly lower than that of the backcross to Swiss mice, but even the latter had counts much lower than that of the parent Swiss mice.

The survival patterns of the F1, F2, and backcross to STR mice were almost identical. These mice lived longer, as a whole, than either parent. The backcross to Swiss mice was somewhat shorter-lived than the other experimental mice; lived about as long as the STR parent, but longer than the Swiss parent.

In general, male mice had lower parasitemias than female mice.

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# COMPARATIVE STUDY OF *SCHIZOTRYPANUM CRUZI* CHAGAS, 1909 AND *S. VESPERTILIONIS* (BATTAGLIA, 1904) FROM COSTA RICA

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In a previous note (Zeledón and Vieto, 1957) we referred to the presence of *Schizotrypanum vespertilionis* in the Costa Rican bats *Glossophaga soricina leachii* and *Hemiderma perspicillatum aztecum*. In the present paper, we report a comparative morphological study of that interesting flagellate and 2 strains of *S. cruzi* from Costa Rica. We shall also discuss the identity of the strains of *Schizotrypanum* from Chiroptera found in several American countries using data of workers who have furnished adequate published information. Appreciation is expressed to Dr. Clark P. Read for advice in the preparation of the manuscript.

## MATERIALS AND METHODS

From 1 of the bats (*Glossophaga s. leachii*) with a high blood infection we obtained several smears which were stained first with Wright's stain and then with Giemsa's stain for at least 12 hours. From these smears we drew 50 trypanosomes, with the aid of a camera lucida and a microscope having a magnification of 2500 diameters. The same procedure was carried out with *S. cruzi*, using a blood smear from a mouse with a heavy parasitemia of a strain isolated in the laboratory in 1952 from *Triatoma dimidiata* and maintained in mice ("R" strain), and blood smears containing another strain isolated from another insect of the same species and maintained in guinea pigs ("C" strain).

Using the procedure of Dias and Freitas (1943) we made measurements of the drawings with the aid of a "Derby" curvimeter (made in Switzerland) having a centimeter scale. All drawings and measurements were performed by one of us (R.Z.) using the same equipment at all times. The nuclear index referred to below is the ratio of the distance from posterior to the center of the nucleus (PN) divided by the distance from the center of the nucleus to the anterior extremity (NA) as defined by Dias and Freitas (1943).

## RESULTS

*S. vespertilionis* is in general smaller than *S. cruzi* (average length: 16 microns for the former and about 21 for the latter) and has a very characteristic position of the nucleus. Thus, *S. vespertilionis* has an average nuclear index of 2.7 in contrast to an average nuclear index of 1.5 for the *S. cruzi* strains which were very similar in their morphological characteristics and measurements. In Table I we present details of the measurements for each of the trypanosomes studied.

TABLE I. *Comparative measurements of the* Schizotrypanum *in microns.*

| Species | PN | | | NA | | | Flagellum length | | | Total length | | | PN/NA | | | No. measured |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | S.D.* | Mean | S.D.* | |
| *S. vespertilionis* | 5.6 | 8.1 | 6.8 | 2.5 | 3.1 | 2.6 | 5.0 | 8.7 | 6.4 | 13.7 | 20.0 | 16.0 | 1.13 | 2.7 | 0.24 | 50 |
| *S. cruzi* "R" | 6.8 | 8.7 | 7.7 | 4.3 | 6.2 | 5.2 | 5.6 | 10.0 | 8.1 | 17.5 | 24.3 | 21.4 | 1.47 | 1.5 | 0.14 | 50 |
| *S. cruzi* "C" | 6.2 | 9.3 | 7.9 | 3.7 | 6.8 | 5.3 | 6.2 | 11.2 | 8.1 | 17.5 | 25.0 | 21.5 | 1.84 | 1.5 | 0.12 | 50 |

* Standard deviation

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It may be seen that the mean nuclear indices of the 2 strains of *S. cruzi* are identical and are significantly different from the mean nuclear index of *S. vespertilionis*. In the case of *S. cruzi* the nuclear index varied from 1.2 to 1.8 and in *S. vespertilionis* varied from 2.2 to 3.2.

Study of the variation in total length of the flagellates showed that, while the smallest specimens of *S. cruzi* may be smaller than the longest of *S. vespertilionis*, the average lengths or the modal patterns of the 2 species are plainly separated.

In Table II we give the differential characteristics of the 2 species of flagellates, based on our personal experience and utilizing the literature available on the subject. We were unable to demonstrate important differences in the culture forms.

TABLE II. *Comparison of the characteristics of* S. vespertilionis *and* S. cruzi.

| Character | *S. vespertilionis* | *S. cruzi* |
|---|---|---|
| Average lengths | 14.6–16.0 microns | 20–21.5 microns |
| Nucleus position | Very anterior | Slightly anterior |
| Average nuclear indices | 2.6–2.7 | 1.4–1.6 |
| Laboratory animal inoculation | Negative | Positive |
| Experimental infection in triatomid bugs | Negative | Positive |
| Pathogenicity | Apparently non-pathogenic | Sometimes very pathogenic (Chagas' Disease) |
| Natural vector | Bat mites (*Leiognathus* spp.)*, bat bug (*Cimex pipistrelli*), bat fleas, (sp.)*, Nycteribiidae (*Listropoda* sp.)*, triatomid bug (*Cavernicola pilosa*) | Triatomid bugs |
| Natural hosts | Several spp. of bats | Several spp. of mammals including bats |
| Geographic distribution | Cosmopolitan | American Continent and Java |

* No experimental confirmation, only flagellate forms in the arthropods.

## DISCUSSION AND CONCLUSIONS

It is evident that accurate biometric study differentiates *S. cruzi* and *S. vespertilionis*. The nuclear indices are very close to the reported indices for the same species of flagellates from other countries. Dias (1940) found a nuclear index of 2.6 for *S. vespertilionis* and 1.6 for a human strain of *S. cruzi* from Brazil. Floch and Lajudie (1943) reported nuclear indices of 1.4 and 1.5 for strains of *S. cruzi* from French Guiana. From the figures presented by Streber (1950) for a Mexican strain of *S. cruzi* we can deduce a nuclear index of 1.4.

We can conclude that the average nuclear index is a quite constant character in the strains studied by several workers and may be taken as an important and fundamental differential character between the 2 species. However, it does not tell us much about the significance of this character in strains from bats in which the average values differ slightly. However, some strains with an average nuclear index less than that for typical strains of *S. cruzi*, such as the strain from *Phyllostomus hastatus* (Dias, 1940; Dias and Pifano, 1941) and from *P. hastatus* and *Artibeus jamaicensis* in French Guiana (Floch, Lajudie and Abonnenc, 1942), have the biological behavior of *S. cruzi* of low virulence. Low virulence is also characteristic of the *Schizotrypanum* isolated from marsupials of the genus *Didelphis*. For this reason Floch and Lajudie (1943) regarded all these trypanosomes as strains of *S. cruzi* that have lost virulence by adaptation to certain hosts, the loss of virulence being accompanied by a migration of the nucleus toward the posterior part of the body of the parasite. Nevertheless we think that the depression in the virulence is specifically related to the environment furnished by the vertebrate host and is essen-

tially independent of the morphological variation. This view is supported by observations on our strain "C" of *S. cruzi* which has the usual nuclear index and low virulence for guinea pigs. It will be of interest to obtain measurements on typical *S. cruzi* strains from different sources from different countries, to shed further light on the nature of variations in the morphology of *S. cruzi*.

If one of the *Schizotrypanum* with a low nuclear index represents a different species, it will probably be that found by Dias and Pifano (1942) in Venezuela in *Phyllostomus elongatum;* this form has a posterior nucleus (nuclear index: 0.8) and attains a maximum length of 29.2 microns. The other trypanosomes reported from bats fit very well in 1 of the 2 species studied in this paper. We consider as *S. cruzi* the following flagellates: The one from *Eumops bonariensis beckeri* of Dias and Romaña (1939); Cartaya's *Trypanosoma phyllostomae* and the "phyllostomae" strain of Dias and Pifano (1941); the Venezuelan trypanosome of Pifano and Dias (1942) and the bat Panamanian trypanosomes of Clark and Dunn (1932). Typical forms of *S. vespertilionis* are the flagellates from *Lonchoglossa ecaudata* and *Carollia perspicillata* (= *Hemiderma perspicillatum*) of Dias (1940), those of several bats from Belem and from "Marajo" Island in Brazil reported by Dias, et al (1942) and those from *Tadarida brasiliensis* and *Histiotus laephotis* reported by Romaña (1945) from Argentina. The *Schizotrypanum* of Romaña and Toranzos (1947), found in bats of the genus *Eptesicus*, are not very well defined; they have a nuclear index resembling that of *S. cruzi* and exhibit biological properties of *S. vespertilionis*. This would merit reinvestigation.

## SUMMARY

A biometric study is reported of *Schizotrypanum cruzi* (2 strains) and *S. vespertilionis* isolated from bats caught near San José, Capital of Costa Rica. For *S. vespertilionis* the nuclear index was 2.7 and the total average length was 16.0 microns and for 2 strains of *S. cruzi* the measurements were 1.5 for the nuclear indices and 21.4 and 21.5 microns for the average total lengths.

A comparative table for both species with different biometric and biological characters is presented.

Some discussion concerning the value of the average nuclear index is offered and a tentative position is given for the *Schizotrypanum* adequately described from bats in our continent.

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## RESEARCH NOTE

### TWO NEW DEFINITIVE HOSTS FOR THE EYE WORM, *THELAZIA CALIFORNIENSIS* PRICE 1930

An eye worm of the genus *Thelazia* was first reported in the United States by Allerton (1929, N. Amer. Vet. **10**: 56) from material removed from the eyes of a dog near Los Angeles, California. Price first recognized the worm as a new species and proposed the name *Thelazia californiensis* (1930, J. Parasitol. **17**: 112–113).

During the summer of 1957, with the cooperation of the California Department of Fish and Game, some areas of Riverside, San Bernardino, Los Angeles, and Inyo counties were investigated for the presence of wild animals infected with the eye worms. The animals examined included 154 jack rabbits, 53 bobcats, 52 coyotes, 12 foxes, 10 cotton-tail rabbits, 6 ground squirrels, 5 raccoons, 3 skunks, 2 badgers, and 1 opossum.

Two of 4 jack rabbits (*Lepus californicus*) and 2 of 6 coyotes (*Canis latrans*) examined a few miles north of Banning were infected. Two of 6 jack rabbits and 5 of 39 coyotes examined near Anza were also infected. Both of these towns are in Riverside County, California. One jack rabbit examined in the mountains at Barton Flats, a few miles north of Mt. San Gorgonio, in San Bernardino County was negative, whereas one of 2 coyotes examined in this area was positive. Two of 23 jack rabbits examined in Cajon Canyon, about 21 miles north of San Bernardino in San Bernardino County, California, were infected. No coyotes were examined in this area. One jack rabbit of 14 examined, about 10 miles north of Lone Pine in Inyo County, California, was infected. No coyotes were examined in the area.

One jack rabbit and 5 coyotes were examined in the mountains about 8 miles south of Banning, but no infections were found. Jack rabbits examined in other areas were free of the worm. The other animals listed above were found in some of the areas mentioned but no eye worms were found in any of them.

One of the members of the Department of Fish and Game, upon seeing the worms removed from a coyote, reported identical worms in the eyes of silver foxes which he raises on his farm at Oak Glen, about 20 miles east of San Bernardino. One of these foxes, a male, examined in the spring of 1958, had 5 worms in its eyes. The other foxes, females heavy with young, were not examined.

The worms removed from the male were examined and found to be *T. californiensis*. This constitutes the first report of this worm in jack rabbits and foxes, although it has been reported in 8 other mammalian hosts.—HOWARD S. BURNETT AND EDWARD D. WAGNER, *School of Tropical and Preventive Medicine and Department of Microbiology, School of Medicine, College of Medical Evangelists, Loma Linda, California.* [The above is a report of part of the findings made under U. S. Public Health Service Grant E-951(C2).]

# *COOPERIA MCMASTERI* AND *COOPERIA SURNABADA* IN THE UNITED STATES*

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While making an examination of nematode parasites recovered at post-mortem from a bovine that originated in Arizona, we found 3 *Cooperia* males which we have identified as *Cooperia mcmasteri* Gordon, 1932. This species was originally described from a calf in Australia. There are no published records of its occurrence in the United States. Baylis (1938) recorded it from sheep in England and referred to reports of its occurrence in both sheep and cattle in Australia and in sheep in New Zealand. It was recorded from central Europe for the first time by Zarnowski (1949), who found it in sheep in Poland. Patyk (1956 a, b) recorded it from cattle in the same country. Recently, Morgan and Soulsby (1956) recorded it for the first time from cattle in England.

*Cooperia mcmasteri* is very similar to, if not identical with, *Cooperia surnabada* Antipin, 1931, which was described from cattle in Russia and was reported from cattle and sheep in Argentina by Ault (1943) and from cattle in Wyoming by Honess (1957).

Both *C. mcmasteri* and *C. surnabada* are characterized by having relatively straight, slender spicules whose distal ends are capped by hyaline membranes the shape of which may be described as triangular, slipper-shaped, or spear-shaped. Also, both species are characterized by an unusually short stem or trunk of the dorsal ray of the bursa. These characters are illustrated in Figure 1. The 2 species are thus set off from the other members of the genus.

There is disagreement as to whether *C. mcmasteri* is a synonym of *C. surnabada*. LeRoux (1936) considered it so, as did Ault (1943). However, Travassos (1937) recognized both species and considered LeRoux's action premature. Baylis (1938) stated that the 2 species should be regarded as distinct pending contrary evidence based on examination of the types. He evidently considered the principal differences between them to be the form of the genital cone and the shape and length of the spicules. Baylis studied specimens supplied by Gordon and found the spicules to vary in length from 0.250 to 0.290 mm as compared with a published range for *C. surnabada* of 0.228 to 0.235 mm. Skrjabin et al (1953) listed both species. Skrajabin, Shikhobalova, and Shul'ts (1954) also recognized both species and agreed with the view expressed by Travassos (1937).

Zarnowski (1949) observed that his specimens had spicule lengths ranging from 0.202 to 0.248 mm, which would place at least some of them within the pre-

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* This work was done at the State College, New Mexico, field station, in cooperation with the New Mexico Agricultural Experiment Station.

** Present address: Coastal Plain Experiment Station, Tifton, Georgia. The writers are indebted to Mrs. Lea Weiselman, Mrs. Eugenia Artschwager, and Dr. Ferdinand Quinones for assistance in translating Polish, Russian, and Spanish references, respectively, and to colleagues in the Animal Disease and Parasite Research Division, A.R.S., Beltsville, Maryland, for assistance in locating references and unpublished records pertaining to these species.

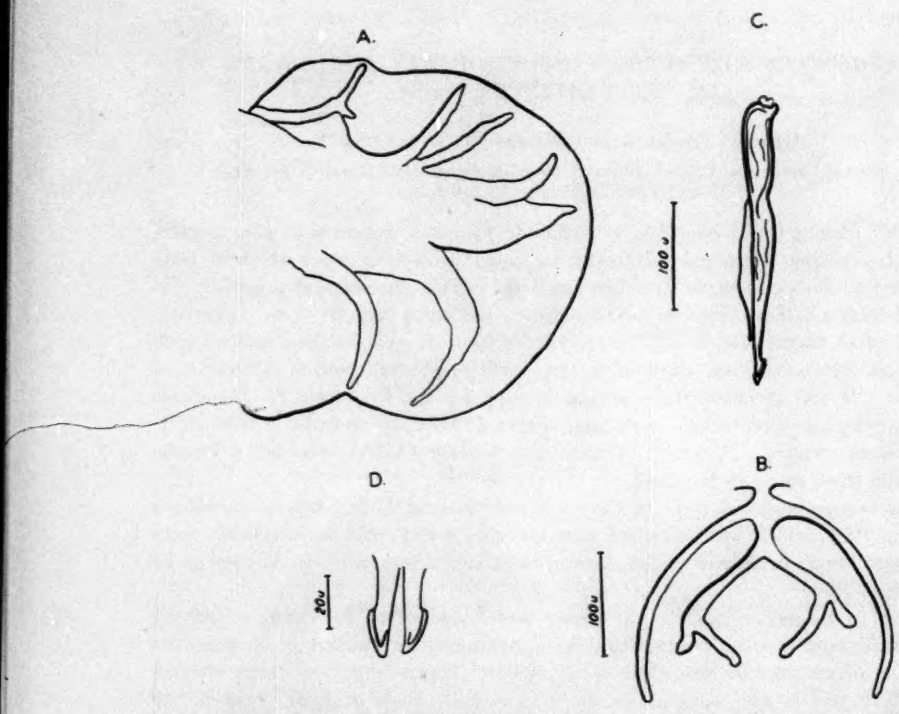


FIGURE 1. Diagnostic characters of *C. mcmasteri* from an Arizona bovine. A.—Rays of bursa; B.—Dorsal and externo-dorsal rays of bursa; C.—Entire spicule; D.—Distal ends of spicules. Made with the aid of a camera lucida.

scribed limits for *C. surnabada*. Nevertheless, he designated his specimens *C. mcmasteri*, noting without comment that LeRoux (1936) considered them synonyms. Ault (1943) found a spicule length range of 0.230 to 0.270 mm in his specimens. He apparently considered the differences unimportant and so designated his specimens as *C. surnabada* because of the priority of that name. In an effort to throw more light on the question of the synonomy of these species, we attempted to borrow type specimens of each. A letter addressed to Dr. D. N. Antipin in Moscow failed to bring a reply. However, Dr. Hugh McL. Gordon kindly sent to us from Australia a mixed collection of *C. mcmasteri*, *C. oncophora*, and *C. punctata*. We have examined 5 of the *C. mcmasteri* specimens and have found the spicules to range in length from 0.225 to 0.261 mm; measurements for 4 of the 5 specimens ranged from 0.225 to 0.234 mm. Thus the majority examined had spicule lengths falling in the range ascribed to *C. surnabada*. The genital cones of these specimens as well as those of the Arizona specimens are, in our opinion, very similar to, if not identical with, the cone of *C. surnabada* as illustrated by Antipin (1931). Although these observations tend to suggest that *C. mcmasteri* and *C. surnabada* are identical, we concur with the view of Baylis (1938) that a comparison of the types ought to precede a definite conclusion. Since the specimens we collected from a yearling from

Arizona (see Table 1) have spicules with lengths that fall within the range reported

TABLE I—*Measurements*[1] *of Arizona specimens compared with those originally reported for* C. mcmasteri *and* C. surnabada *(in millimeters).*

| | *C. surnabada* Antipin, 1931 | *C. mcmasteri* Gordon, 1932 | Arizona specimens |
|---|---|---|---|
| Total length | 4.989–5.470 | 6.000–8.000 | 7.200–9.920 |
| Maximum width | 0.243–0.296 | 0.160–0.200 | 0.150–0.180 |
| Width of head | 0.038 | Not given | 0.039–0.042 |
| Length of esophagus | 0.405–0.410 | 0.400 | 0.429–0.442 |
| Length of stem of dorsal ray | 0.077[2] | 0.070–0.090 | 0.060 (approx.) |
| Length of spicules | 0.228–0.235 | 0.270[3] | 0.267–0.279 |

[1] Males only.
[2] This measurement estimated from Antipin's illustration.
[3] Baylis's measurements of specimens loaned by Gordon ranged from 0.250 to 0.290 mm.

by Baylis for *C. mcmasteri,* we are listing them as that species.

Although the occurrence of *C. mcmasteri* in this country has not, so far as we know, previously been reported and that of *C. surnabada* in American cattle was reported only very recently (Honess, 1957), the distribution of these species in the United States is not limited to Wyoming and Arizona. Recently, one of us (W. W. B). collected from cattle in Georgia and Florida specimens which he has identified as *C. mcmasteri*; their spicules exceeded in length the range reported for *C. surnabada.* The following other previously unpublished records have been brought to our attention.

Mr. John T. Lucker (personal communication, 1957), of the Animal Disease and Parasite Research Division, recently had occasion to examine a lot of *Cooperia* (U.S.N.M. 24516) which had been collected from *Bos taurus* and forwarded on November 27, 1920, to the then Zoological Division of the Bureau of Animal Industry by Dr. L. H. Evans of Essex Junction, Vermont. These parasites had been identified as *C. oncophora* by Dr. M. C. Hall. Mr. Lucker found the lot to contain, in addition to *C. oncophora,* males which could be identified as either *C. surnabada* or *C. mcmasteri.*

Dr. J. R. Douglas (personal communication, 1956) informed us of the collection of *C. mcmasteri* from cattle in Oregon and from deer (*Odocoileus hemionus columbianus*) in California; this identification of 3 specimens (U.S.N.M. 55700) from the former host was confirmed by Mr. Lucker.

In 1948, Dr. D. A. Porter (personal communication, 1957) collected and identified specimens of *C. surnabada* from a bovine in Georgia. These specimens are no longer available. However, this species was identified in other collections obtained from cattle, also from Experiment, Georgia, between 1946 and 1949. Three of the specimens from this locality were recently examined by Mr. Lucker, who found the length of their spicules to be within the range reported for *C. mcmasteri.* We have examined 4 of the specimens from Vermont which were selected from U.S.N.M. 24516 by Mr. F. W. Douvres of the ADP Division, 2 of Douglas's specimens from Oregon (U.S.N.M. 55700), and 2 of Honess's specimens from Wyoming (U.S.N.M. 55701) whose identification was confirmed by Mr. Douvres. The Vermont specimens had spicules ranging in length from 0.228 to 0.252 mm. The spicules of the Oregon specimens measured 0.270 mm. The Wyoming specimens had spicules measuring from 0.243 to 0.258 mm. If one accepts the ranges stipulated by Baylis for *C. mcmasteri* and by Antipin for *C. surnabada,* both species would be present in

the Vermont and Wyoming material, and both of the Oregon specimens examined would be classed as *C. mcmasteri*.

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## RESEARCH NOTE

### IMPROVEMENT OF A DEVICE FOR PURIFYING BALANTIDIA

Recently a description of an improved device for purifying balantidia was published in this journal (1958, **44**: 126–127). An improvement of the device was worked out after that paper went to press.

The apparatus previously described consisted of separation units attached to a glass plate with scotch tape. In the new arrangement, 6 of the separation units are held together by a rack consisting of a glass plate 38 × 17.5 cm (C) to which are cemented two plexiglass retaining plates with openings near the top and bottom for holding the separation units in place. The units and the methods of handling the ciliate-containing materials are the same as previously described.—SERHIJ KRASCHENINNIKOW, *Zoological Laboratory, University of Pennsylvania, Philadelphia, Pa.*

# THE EARLY INDUCTION IN COTTON RATS OF IMMUNITY TO THEIR FILARIAL WORMS*

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Previous experiments (Olson et al, 1955) have shown that the natural immunity of the white rat to *Litomosoides carinii*, a filarial parasite of the cotton rat, is not effective against worms which have already developed 7 days in the cotton rat. The present experiments were undertaken to determine whether the acquired immunity of the cotton rat which we have described (Macdonald and Scott, 1953) is also restricted to the first 7 days of development. Preliminary reports of these experiments have been presented by Scott et al (1955) and Macdonald and Scott (1957).

## MATERIALS AND METHODS

The cotton rats were immunized by the subcutaneous introduction on successive occasions of infective larvae which had been dissected from tropical rat mites according to the methods described by Scott and Macdonald (1953). To provide worms for transfer, infective larvae were similarly introduced into donor cotton rats. Seven days later the developing worms were washed from the pleural cavity in saline solution. They were examined rapidly to ascertain that they were all in the same stage of development, counted and flushed through an incision into the abdominal cavity of the recipient cotton rats. The incision was closed in 2 layers and at autopsy only a minimum of scar tissue and no evidence of bacterial infection was observed. At the termination of the experiments the worms were removed from the pleural cavity in saline solution, fixed in hot alcohol, mounted in glychrogel and measured by projection.

Differences between the means of the length of the worms were tested for statistical significance by the one-tailed "t" test (Dixon and Massey, 1951). Differences between percentages starting to molt were tested by Fisher's Exact Method or, where the numbers were sufficiently large, by one-tailed probability values from the Chi Square Test with Yates' correction (Fisher, 1932).

Some of the cotton rats were from our laboratory-bred colony of *Sigmodon hispidus texianus* and the worms with which they were infected were originally derived from the same strain of wild cotton rats. Other cotton rats were from the Tumblebrook Farms colony of *S. hispidus hispidus* and were infected with worms of a strain originally derived from cotton rats of the same subspecies. For each individual experiment 1 strain of rats and the corresponding strain of worms was used throughout.

## RESULTS

### *First series*

The first series of experiments was designed to determine whether the immunity which retards the growth and development of worms of a superimposed infection introduced as infective larvae, also affects worms introduced after 7 days' development in a donor cotton rat. Half of the recipient cotton rats were immunized by infective larvae given subcutaneously on three occasions at intervals of 5 or 6 days. The total number of larvae given and the number subsequently recovered from each rat is shown in Table I. From 32 to 36 days after the final immunizing infec-

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TABLE I—*Growth and development of worms transferred after 7 days' development in previously unexposed cotton rats to cotton rats immunized by 3 previous infections in comparison with worms transferred to unimmunized control rats.*

| Immunizing infections | | 7-day worms transferred | | | | | |
|---|---|---|---|---|---|---|---|
| Number larvae given | Number worms recovered | Number introduced | % recovered | Mean length ± S.E. in tenths of millimeters (number measured) | | % at least starting final molt | |
| | | | | Males | Females | Males | Females |
| 192 | 95 | 39 | 38 | 73 ± 12( 4) | 96 ± 6( 8) | 83 | 67 |
| Control | | 43 | 74 | 70 ± 2(17) | 105 ± 4(15) | 100 | 73 |
| 161 | 85 | 41 | 59 | 65 ± 3(12) | 96 ± 5(12) | 83 | 50 |
| Control | | 42 | 19 | 76 ± 3( 3) | 94 ± 10( 5) | 100 | 40 |
| 144 | 25 | 42 | 76 | 68 ± 3(19) | 103 ± 5(13) | 68 | 0 |
| Control | | 43 | 63 | 75 ± 4(14) | 105 ± 4(12) | 86 | 38 |
| 169 | 60 | 39 | 54 | 68 ± 2(12) | 101 ± 4( 9) | 83 | 22 |
| Control died | | | | | | | |

tion, worms which had been developing for 7 days in previously uninfected donor cotton rats were transferred surgically into the abdominal cavity of the recipient rats. Approximately half of the worms from each donor rat were given to one of these immunized rats and half to a previously uninfected control rat as shown in the table. The rats were killed 17 days after the transfer, i.e. 24 days after the transferred worms had originally been introduced into the donor rats. The table also shows the percentage of transferred worms recovered at autopsy and bears out our previous experience that percentage recovery is not a good measure of immunity. On the average the percentage is about equal to that observed with infections established by infective larvae. The most important criteria are the mean length of the worms and the percentage which had at least started the final molt. The figures in the table show that on the basis of these criteria there are no obvious differences between the immunized and the control series.

To verify these conclusions statistically the length measurements were added for all worms of each sex from each type of rat. Means for the pooled values obtained are shown in Table II. The numbers of worms molting were also added in a

TABLE II—*Comparison of pooled values of mean length of transferred worms and percentage at least starting final molt for immunized and non-immunized recipients.*

| | Mean length of worms ± S.E in tenths of millimeters | | % at least starting final molt | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Control rats | 73 ± 2 | 103 ± 3 | 94 | 55 |
| Immunized rats | 68 ± 2 | 99 ± 2 | 78 | 33 |
| Difference | 5 | 4 | 16 | 22 |

similar manner and percentages calculated from the pooled values. The differences between the groups shown in the table were all found to show no statistical significance even at the 5% level.

As far as can be determined from these experiments there is no evidence that worms which had developed 7 days in previously uninfected rats were retarded in growth or development when transferred to immunized rats, i.e. rats with preexisting infections, for an additional period of 17 days.

*Second series*

In the second series of experiments worms which had developed for 7 days in

immunized cotton rats were transferred to unimmunized cotton rats for a further 17-day period of development. The donor rats were given from 50 to 120 infective larvae subcutaneously on each of two occasions 7 to 10 days apart, the average number given each time being 87. From 30 to 60 days after the second infection, they were infected the third time with 105 to 130 larvae subcutaneously. Seven days later the animals were killed and the worms removed in saline. There was no difficulty distinguishing the younger worms from those of the immunizing infections. The 7-day-old worms from each rat were introduced into the abdominal cavity of another cotton rat which had never been previously exposed to infection.

At the time that the above-mentioned donor rats were given their final infection 2 additional groups of cotton rats were infected for the first time with larvae from the same lot of mites. At the end of 7 days the worms from one of these latter groups were transferred to the abdominal cavity of previously unexposed rats. The worms of the other group were allowed to remain in the same hosts. All rats were killed 17 days after the transfer operation, i.e. 24 days after the worms had been introduced as infective larvae.

Thus at the end of the experiment there were 3 groups of worms each of which had been in one or more rats for a total of 24 days. The worms of the first group were in previously infected rats for 7 days and then in previously uninfected rats for 17 days, those of the second group were in previously uninfected rats for 7 days and then in a second series of uninfected rats for 17 days, and those of the third group remained in the same previously uninfected rats for the entire 24 days. The measurements and determinations made on these worms are shown in table III.

TABLE III—*Recovery and measurements at 24 days of worms transferred to previously uninfected cotton rats after 7 days' development in previously infected cotton rats (Immunized) or in previously uninfected cotton rats (Unimmunized), in comparison with worms developing without transfer in previously uninfected cotton rats (Control).*

| | Number of rats | Mean number introduced | Mean % recovered | Mean length in tenths of millimeters | | % at least starting final molt | |
|---|---|---|---|---|---|---|---|
| | | | | Males | Females | Males | Females |
| Immunized | 5 | 38 | 56 | 55 | 75 | 27 | 0 |
| Unimmunized | 5 | 51 | 60 | 62 | 89 | 69 | 7 |
| Control | 5 | 111 | 38 | 64 | 90 | 74 | 16 |
| Differences: Control minus Unimmunized | | | | 2 | 1 | 5 | 9 |
| Control minus Immunized | | | | 9** | 15** | 47** | 16** |
| Unimmunized minus Immunized | | | | 7** | 14** | 42** | 7* |

* Indicates a difference significant at the 5 per cent level.
** Indicates a difference significant at the 1 per cent level.

The data were pooled for all worms from all rats treated alike.

The criteria used to determine the effects of the different treatments were the same as in the first series of experiments. It will be noted from the table that there were no significant differences between the controls and the unimmunized. The worms of the immunized group were, however, significantly shorter than those from either the unimmunized group or the control group, and the percentage at least starting to molt was significantly lower.

The obvious conclusions are that transferring worms from one unimmunized rat to another unimmunized rat did not appreciably retard their growth. The retardation of growth seen in those transferred from immunized rats to unimmunized rats apparently resulted from their being for 7 days in the rats which had previous

infections. Measurements of another group of worms which had been in a previously unexposed rat for 7 days showed that at 7 days they averaged 1.1 mm long while infective larvae average 0.83 mm. It is obvious, then, that very little of the retardation seen in worms which were transferred from immunized rats to unimmunized rats could have occurred in the first rat. Whatever the mechanism of retardation is, its effect must have been produced during the first 7 days, but the results of this effect appeared primarily after the first 7 days.

## DISCUSSION

Infective larvae of *Litomosoides carinii* average about 0.8 mm in length. In previously uninfected rats they increase during the first 7 days to about 1.1 mm in length. Previous experiments (Macdonald and Scott, 1953) have shown that during the first 24 days the male worms increase to an average of 8.8 mm and the female worms to an average of 10.9 mm. On the other hand at the end of the first 24 days in previously infected rats the males average only 4.0 mm and the females only 4.9 mm (Macdonald and Scott, 1953). It was not practical to attempt to measure whether any of this retardation of growth occurred during the first 7 days, but it is obvious that most of it must have occurred during the subsequent 17 days.

The present experiments show that if worms are in previously uninfected rats for the first 7 days and are then transferred to previously infected rats their growth between the 7th and 24th days is not retarded by the presence of the previous infection. If, on the other hand, the worms spend the first 7 days in previously infected rats and are then transferred to previously uninfected rats their growth during the subsequent 17 days is retarded nearly as much as if they had been in previously infected rats for the entire 24 days.

It seems clear that the retarding effects of pre-existing infections act primarily on worms during their first 7 days of development in the definitive host. The effects of this action are expressed, however, principally during the subsequent 17 days. The surprising thing is that this retarding effect is carried over by the worms even when they are transferred to non-immunized hosts.

## SUMMARY

The presence of previous infections of the filarial worm, *Litomosoides carinii*, in cotton rats retards the growth of worms of subsequent infections during at least the first 24 days of their development in the definitive host. Worms which develop 7 days in previously uninfected cotton rats and are then transferred surgically to previously infected cotton rats are not retarded in growth during the subsequent 17 days. Worms which develop 7 days in previously infected cotton rats and are then transferred to previously uninfected cotton rats show retardation of growth during the 17-day period after transfer. It seems obvious that the retarding effect is produced during the first 7 days of development, but is expressed principally during the subsequent 17-day period.

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## RESEARCH NOTE

### *SARCOCYSTIS RILEYI* IN SAGE GROUSE

Parasitism of grouse by *Sarcocystis* has not previously been noted (Erickson, 1940, Auk. **57**: 4 : 514–519), but the parasite has been found in domestic fowl and more recently by Clapham (1957, Nature **180**: 1294) in the red-legged partridge, *Alectoris rufa*, and the pheasant, *Phasianus colchicus*, in the British Isles. Specimens of sage grouse, *Centrocercus urophasianus*, taken in southern Alberta in early March, 1955, were found infected with a species of *Sarcocystis*. The parasite was not observed macroscopically but became apparent during histological studies of striated muscle removed from the breast and legs. Cysts containing large numbers of spores of the protozoan were found embedded in the muscle fibers. The cysts were worm-like structures, the largest of which was approximately 1 mm long and 50 microns in diameter. Although completely surrounded by myofibrils, sarcoplasm, and the sarcolemma of the muscle fiber, each cyst has its own wall, a very fine membrane supported on the outside by longitudinal rods which give a feathered appearance to its cross-section. The arrangement of the spores within the cyst is not orderly.

Small pieces of tissue were taken from the pectoralis major, the pectoralis minor, and the sartorius muscle of 2 sage grouse, one an adult male, the other an immature male estimated to be about 9 months of age. *Sarcocystis* was found in all samples except that taken from the sartorius muscle of the adult. The immature bird appeared to be more heavily infected than the adult. The 2 sage grouse were taken near Wildhorse, in extreme southeastern Alberta, from a flock of about 75 grouse. While these 2 comprise only a small sample it would appear that the prevalence of *Sarcocystis* is high in sage grouse in Alberta. No deleterious effect of this parasitism has been noted in these birds. This is in keeping with observations on parasitism by *Sarcocystis* in other birds and in mammals.

Muscle from nearly all species of gallinaceous birds occurring in Alberta has been examined microscopically during the course of this investigation but no other *Sarcocystis* infection was noticed. However, 3 instances of *Sarcocystis* in the mallard, *Anas platyrhynchos*, in the Edmonton area have come to my attention. In each case infection was heavy and the cysts were much larger than those in the sage grouse, being readily visible to the unaided eye. Dr. Marshall Laird, MacDonald College, Quebec, who has kindly examined sections of the muscle of the sage grouse and a mallard, identifies the parasites in each as *Sarcocystis rileyi*.—W. RAY SALT, *University of Alberta, Edmonton, Canada.*

# NOTES ON *ASCARIDIA DISSIMILIS*

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Several years ago we were puzzled by the difficulties which turkey growers were reporting to us with regard to the treatment of turkeys for large roundworm infections with nicotine-containing products. The same product which was quite effective in the removal of such worm infections from chickens was, according to field reports, much less effective in removing these worms from turkeys. At one of the annual meetings of the American Society of Parasitologists, this problem was discussed with Dr. Paul Harwood and it was learned that he was having a similar experience. It was decided that the species of large roundworm infecting turkeys should be determined to make certain that we were dealing with the same species of worm in both the chicken and turkey.

Arrangements were made to obtain specimens of large roundworms from turkey flocks in the midwestern area of the United States. Identifications of the worms were based on the description of the location of the papillae in the posterior end of the male worm as figured by Wehr (1940) and subsequently by Horton-Smith and Long (1957). During the course of this work, Dr. Holger Madsen visited our Laboratories and pointed out to us the difference in the shape of the spicules of *Ascaridia galli* and *A. dissimilis*.

Specimens of large roundworms from turkey flocks located in Minnesota, Iowa, Indiana, Kansas and from 2 flocks in Texas, were examined. In every case the male worms fitted the description of *A. dissimilis* using the location of the papillae as the means of identification. Horton-Smith and Long (1957) reported the finding of this species in turkeys in Britain. These authors state that Wehr, in a communication to them, made the statement that *A. dissimilis* is the predominant species in turkeys in the vicinity of Washington, D. C. Wehr (1942) reported the finding of this species in wild turkeys from Georgia and Pesnnsylvania.

Since the posterior portions of these worms had been saved, it was possible to follow the suggestion of Dr. Madsen and compare the shape and length of the spicules found in these worms with those found in our strain of *A. galli*. The spicules for the 2 species are different as shown in Figure 1. The spicules of *A. galli* (Fig. 1, A) are almost twice as long and the angle of the funnel portion is less acute than those of *A. dissimilis* (Fig. 1, B). Subsequent identifications were made using both criteria, position of the papillae and shape and length of spicules.

In the first attempt to establish the life-cycle of *A. dissimilis* in the laboratory, infective material pooled from several worms was used. This infective material was administered to both poults and chicks which were about 2 weeks old. When these birds were killed some 6 weeks later, only *A. dissimilis* was found in the poults and *A. galli* in the chicks.

The worms recovered from the above-mentioned birds were discarded and a single female worm from a field infection was selected. Using infective material de-

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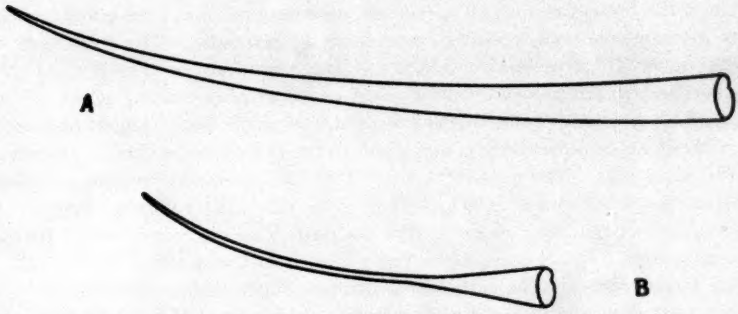


FIGURE 1. A. Spicule of *A. galli;* B. Spicule of *A. dissimilis.*

rived from this worm, a "strain" of *A. dissimilis* has been established which is the source of all subsequent infections used in our laboratory.

Infections of both chickens and turkeys were attempted with this strain of worm. Ten chickens were each given 105 embryonated eggs from a single female worm. These chickens were killed and examined 4 weeks later. No worms were found. Six chickens and 6 turkey poults were each given approximately 140 embryonated eggs. One month later the droppings from the turkey poults showed *Ascaridia* ova on salt flotation. This is much less time required for maturation than reported by Wehr (1942). The 6 chickens were killed 36 days after infection and 1 immature male worm was found. This worm was identified as *A. dissimilis.* The 6 turkeys were killed 45 days after infection and 68, 50, 51, 32, 51, and 51, or a total of 303 worms were recovered. All of the male worms examined were identified as *A. dissimilis.*

Forty White Leghorn roosters, approximately a month old, were each given orally 360 embryonated ova. Forty-seven Beltsville White turkey poults were also given embryonated eggs when they were about 1 month old. The chickens were killed and examined for worms 4, 5 and 7½ weeks after infection. No worms were recovered from these birds. Of the turkeys, 20 poults were used in an anthelmintic test 6 weeks after infection and 19 of these birds harbored 958 worms; 19 additional birds were used in another anthelmintic test 7 weeks after infection and 18 of these birds harbored 293 worms; and 8 birds were used in a third anthelmintic test 8 weeks after infection and none of the poults harbored worms. The results of these tests indicate that the chicken is rather resistant to infection with *A. dissimilis.*

It had been noted in previous work that we had failed to obtain the same degree of infection with *A. galli* in turkey poults that we obtained in chickens. To test this observation, embryonated eggs were taken from a single female *A. galli* and were given to 6 turkey poults and 6 chickens about 14 days old. Each bird was given approximately 350 embryonated eggs. These 12 birds were killed 36 days later. The chickens harbored 45, 9, 14, 1, 0 and 0 worms; or a total of 68. Twenty-four male worms from this total were identified as *A. galli.* The turkey poults harbored 1, 0, 0, 0, 0 and 7; or a total of 8 worms. Four of these worms were males and were identified as *A. galli.* This comparison, although not by any means extensive or complete, indicates that turkeys, while susceptible to infections with *A. galli,* do not become infected readily with this species.

One of the flocks from which specimens were received had been given approximately 3 treatments with a nicotine-containing anthelmintic. The treatments did not seem materially to reduce the infection. At the time that we received the specimens, we were in the process of developing a piperazine-containing water wormer (Wazine®). A supply of this wormer was shipped to the State Diagnostic Laboratory, with which we were dealing, and given to the grower to be used in accordance with our directions. The grower reported that the piperazine wormer essentially eliminated the problem and at the same time he submitted additional birds to the State Laboratory and they reported that the birds they examined were essentially free from worms.

This field report plus the anthelmintic studies which we have carried out in our Laboratories, show that piperazine in the proper dosage is highly effective in removing maturing and mature *A. dissimilis*. Our experience indicates that a dosage of 100 mg of the piperazine moiety removes at least 90% of mature worms from turkey poults.

These studies indicate that in the United States it is probable that the predominant large roundworm species in turkeys is *A. dissimilis*. Horton-Smith and Long stated in their paper, "Although we have no grounds for suspecting that *A. dissimilis* is the predominant ascarid species in turkeys in this country (England) it is possible that all ascarids hitherto recovered from turkeys have been assumed, incorrectly, to be *A. galli*." We are inclined to agree that the same situation exists in the United States.

A piperazine-containing anthelmintic is the drug of choice for the removal of these worms.

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## RESEARCH NOTE

### FIRST REPORT OF *ORNITHODORUS (OTOBIUS) MEGNINI* FROM THE PRONGHORN ANTELOPE, *ANTILOCAPRA AMERICANA*

Two male pronghorn antelopes, *Antilocapra americana*, which were collected approximately 20 miles north of Roswell, New Mexico, one on January 15, the other on February 15, 1958, were found to be parasitized by ear ticks. Four nymphs were obtained from the first animal and 3 from the second. All of the parasites were situated deep within the external ear canal.

These nymphs were sent to the Animal Parasite Laboratory of the Animal Disease and Parasite Research Division, Beltsville, Maryland, for identification. Mr. Allen McIntosh classified the specimens as the spinose ear tick, *Ornithodorus (Otobius) megnini*. Since, according to Mr. McIntosh, there were no previous records of this tick from the pronghorn antelope in the Index-Catalogue of Medical and Veterinary Zoology, this finding is considered to be the first report of the spinose ear tick from this host. The specimens from the first antelope were deposited in the U.S. National Museum Parasite Collection as No. 38318.

This work was done in cooperation with the New Mexico Agricultural Experiment Station.—G. A. SCHAD, *Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, State College, New Mexico.*

# THE DISTRIBUTION AND PERSISTENCE OF HOOKWORM LARVAE IN THE TISSUES OF MICE IN RELATION TO SPECIES AND TO ROUTES OF INOCULATION*

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Numerous confirmations of Looss' (1896) demonstration of skin penetration by hookworm larvae have established the general view that infections are almost exclusively acquired in this manner. On the other hand, it is now well known that after oral inoculation *Ancylostoma caninum* readily develops in dogs and cats and that development is direct, without migration through the lungs (Foster and Cross, 1934). It has been shown also that after inoculations into mice and other common laboratory hosts, the larvae of *Ancylostoma caninum, A. duodenale* and *N. americanus* enter the tissues and undergo development, or not, in much the same manner whether given orally or percutaneously. In a recent study of the identifying features of the infective larvae and more advanced stages of several species of nematodes in the tissues of mice, Nichols (1956a, b) found that for *A. caninum* and *N. americanus* the location and character of the larvae were generally similar whether inoculations were given by mouth or by skin. No attempt was made, however, to compare the modes of inoculation in this regard or to describe in detail the distribution and persistence of the larvae.

The present investigation was undertaken to examine more closely the migratory behavior and longevity of the larvae of *A. caninum, A. duodenale* and *N. americanus* in relation to different methods of inoculation in the mouse.

## MATERIALS AND METHODS

Month-old mice were inoculated in 1 of 3 ways: By placing larvae in the mouth, by introducing them directly into the stomach by catheter, and by placing them on unbroken skin in moist gauze pads. Three species of hookworms were used, *Ancylostoma caninum, Ancylostoma duodenale* and *Necator americanus*. Larvae for inoculation were obtained from 15-day-old charcoal cultures. The mice were prepared for infection by using ether anesthesia and tying them on animal boards. Five hundred larvae were placed on a small pad of gauze which was then placed in the mouse's mouth. After 30 minutes the gauze pad was removed and any remaining larvae were recovered by the Baermann technic and were then placed under the tongue. In this manner 7 mice were infected with each of the 3 species of larvae. Similarly 500 larvae of each species were administered separately to each of 7 mice by stomach tube, and in a third series of 21 mice, gauze pads were dampened with water containing 500 larvae which were permitted to remain on the shaved groin for 30 minutes. The few larvae that did not penetrate were collected by the Baermann technic and pipetted directly onto the skin. In all cases, the mouse remained motionless until the fluid had dried.

It was estimated that less than 1% of the original 500 larvae were lost in the process of inoculation and that this error was uniformly distributed in the different experimental groups. For this reason, the error was not taken into consideration in computing the percentage of recovery.

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One mouse from each group was sacrificed at 6, 12, 18, 24, 30, 36 and 66 days after inoculation. At sacrifice the body was divided into 5 parts—the lungs, liver, small intestine, contents of the small intestine, and muscle (carcass). In the cases of *A. duodenale* and *A. caninum* the larvae were recovered from all portions excepting intestinal contents and muscles by digestion in artificial gastric juice (0.5% pepsin, 0.7% HCl in saline). The Baermann technic was used to recover larvae from the shredded muscles and the intestinal contents of the above 2 species, and from all portions of the shredded tissues for *N. americanus*. With the muscles were included all portions of the carcass except the skin, tail, feet, kidney, stomach and large intestine. The small intestine was removed to a petri dish, thoroughly flushed with saline to obtain the contents separately and was then cut into very fine pieces, ground in a Waring blendor and digested. From the intestinal contents larvae were collected by the Baermann technic. Some of the free larvae may have remained with the gut tissues and others that were inside the gut tissues may have been forced into the free contents by the handling procedures.

## RESULTS

There was considerable variation in the yields at different times and from different tissues (Tables I–III). It is doubtful, however, if any significance can be attri-

TABLE I. *Number of larvae of* Ancylostoma caninum *recovered from various locations and at various periods after inoculation of 500 larvae by mouth, stomach and skin respectively in mice weighing approximately 25 grams.*

| No.* | Inoculation by | Day | Lung | Liver | Small Intestine | | Muscle | Total | % Inoculum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tissue | Contents | | | |
| 1 | stomach | 6 | 15 | 0 | 2 | 0 | 346 | 363 | 72.6 |
| 2 | mouth | 6 | 60 | 3 | 3 | 10 | 350 | 426 | 85.2 |
| 3 | skin | 6 | 2 | 0 | 0 | 0 | 89 | 91 | 18.2 |
| 4 | stomach | 12 | 1 | 0 | 1 | 1 | 168 | 171 | 34.2 |
| 5 | mouth | 12 | 0 | 0 | 1 | 4 | 147 | 152 | 30.4 |
| 6 | skin | 12 | 0 | 0 | 0 | 0 | 127 | 127 | 25.4 |
| 7 | stomach | 18 | 0 | 0 | 1 | 0 | 287 | 288 | 57.6 |
| 8 | mouth | 18 | 2 | 0 | 0 | 0 | 134 | 136 | 27.2 |
| 9 | skin | 18 | 0 | 0 | 0 | 0 | 106 | 106 | 21.2 |
| 10 | stomach | 24 | 1 | 0 | 0 | 0 | 89 | 90 | 18.0 |
| 11 | mouth | 24 | 0 | 0 | 0 | 0 | 56 | 56 | 11.2 |
| 12 | skin | 24 | 0 | 0 | 0 | 0 | 175 | 175 | 35.0 |
| 13 | stomach | 30 | 0 | 0 | 0 | 0 | 53 | 53 | 10.6 |
| 14 | mouth | 30 | 0 | 0 | 0 | 0 | 12 | 12 | 2.4 |
| 15 | skin | 30 | 0 | 0 | 0 | 0 | 76 | 76 | 15.2 |
| 16 | stomach | 36 | 0 | 0 | 0 | 0 | 48 | 48 | 9.6 |
| 17 | mouth | 36 | 1 | 0 | 0 | 1 | 226 | 228 | 45.2 |
| 18 | skin | 36 | 0 | 0 | 0 | 0 | 101 | 101 | 20.2 |
| 19 | stomach | 66 | 0 | 0 | 0 | 0 | 58 | 58 | 10.2 |
| 20 | mouth | 66 | 0 | 0 | 0 | 0 | 42 | 42 | 8.4 |
| 21 | skin | 66 | 0 | 0 | 0 | 0 | 61 | 61 | 12.2 |

* Nos. 16–21, males.

buted to the differences in total yields from mice inoculated by different routes. Excepting the relatively low recovery of *A. caninum* and *N. americanus* on the 6th day after skin inoculation, and of *A. caninum* 30 days after inoculation into the mouth, results were essentially the same regardless of the mode of inoculation. At 6 days about 1/3 of the *A. duodenale* larvae were recovered in each instance. For *A. caninum* and *N. americanus* yields at 6 days were approximately the same after stomach or mouth inoculations, ¾ and ½ respectively, while cutaneous inoculation gave much lower yields of 1/5 and 1/3 respectively.

The site of entry into the body had an important influence on the distribution of the larvae in the tissues, and the different species differed in their migratory behavior. By the 6th day after inoculation and later, the larvae of all species were found primarily in the muscles, if at all. However, *A. duodenale* occurred frequently

TABLE II. *Number of larvae of* Ancylostoma duodenale *recovered from various locations and at various periods after inoculation of 500 larvae by mouth, stomach and skin respectively in mice weighing approximately 25 grams.*

| No.* | Inoculation by | Day | Lung | Liver | Small Intestine | | Muscle | Total | % Inoculum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tissue | Contents | | | |
| 1 | stomach | 6 | 13 | 36 | 13 | 14 | 106 | 182 | 36.4 |
| 2 | mouth | 6 | 12 | 46 | 6 | 2 | 98 | 164 | 32.8 |
| 3 | skin | 6 | 6 | 3 | 0 | 0 | 134 | 143 | 28.6 |
| 4 | stomach | 12 | 1 | 25 | 0 | 1 | 62 | 89 | 17.8 |
| 5 | mouth | 12 | 0 | 10 | 0 | 3 | 115 | 128 | 25.5 |
| 6 | skin | 12 | 10 | 3 | 0 | 2 | 72 | 87 | 17.6 |
| 7 | stomach | 18 | 0 | 1 | 3 | 0 | 8 | 12 | 2.4 |
| 8 | mouth | 18 | 9 | 8 | 1 | 0 | 5 | 23 | 4.6 |
| 9 | skin | 18 | 3 | 0 | 0 | 3 | 45 | 51 | 10.2 |
| 10 | stomach | 24 | 0 | 0 | 0 | 0 | 5 | 5 | 1.0 |
| 11 | mouth | 24 | 0 | 5 | 0 | 2 | 6 | 13 | 2.6 |
| 12 | skin | 24 | 0 | 3 | 0 | 0 | 38 | 41 | 8.2 |
| 13 | stomach | 30 | 0 | 4 | 0 | 0 | 1 | 5 | 1.0 |
| 14 | mouth | 30 | 0 | 0 | 0 | 0 | 1 | 1 | 0.2 |
| 15 | skin | 30 | 5 | 0 | 1 | 0 | 10 | 16 | 3.2 |
| 16 | stomach | 36 | 0 | 0 | 0 | 2 | 1 | 3 | 0.6 |
| 17 | mouth | 36 | 1 | 0 | 0 | 0 | 18 | 19 | 3.8 |
| 18 | skin | 36 | 0 | 4 | 0 | 0 | 8 | 12 | 2.4 |
| 19 | stomach | 66 | 0 | 0 | 0 | 0 | 5 | 5 | 1.0 |
| 20 | mouth | 66 | 0 | 0 | 0 | 0 | 3 | 3 | 0.6 |
| 21 | skin | 66 | 0 | 0 | 0 | 0 | 11 | 11 | 2.2 |

* All males except Nos. 1–3.

TABLE. III. *Number of larvae of* Necator americanus *recovered from various locations and at various periods after inoculation of 500 larvae by mouth, stomach and skin respectively in mice weighing approximately 25 grams.*

| No.* | Inoculation by | Day | Lung | Liver | Small Intestine | | Muscle | Total | % Inoculum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tissue | Contents | | | |
| 1 | stomach | 6 | 106 | 25 | 0 | 0 | 110 | 241 | 48.2 |
| 2 | mouth | 6 | 140 | 16 | 0 | 0 | 126 | 282 | 56.4 |
| 3 | skin | 6 | 80 | 0 | 0 | 0 | 90 | 170 | 34.0 |
| 4 | stomach | 12 | 7 | 0 | 0 | 0 | 18 | 25 | 5.0 |
| 5 | mouth | 12 | 4 | 0 | 0 | 0 | 13 | 17 | 3.4 |
| 6 | skin | 12 | 0 | 0 | 0 | 0 | 23 | 23 | 4.6 |
| 7 | stomach | 18 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 8 | mouth | 18 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 9 | skin | 18 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 10 | stomach | 24 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 11 | mouth | 24 | 0 | 7 | 0 | 0 | 0 | 7 | 1.4 |
| 12 | skin | 24 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 13 | stomach | 30 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 14 | mouth | 30 | 0 | 0 | 0 | 0 | 1 | 1 | 0.2 |
| 15 | skin | 30 | 0 | 0 | 0 | 0 | 4 | 4 | 0.8 |
| 16 | stomach | 36 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 17 | mouth | 36 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 18 | skin | 36 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 19 | stomach | 66 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 20 | mouth | 66 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 21 | skin | 66 | 0 | 0 | 0 | 0 | 0 | 0 | |

* Nos. 1–6 and 19–21, males.

in the lungs and liver also, and a considerable number were found in the gut and gut contents up to 18 days (Table II). In 4 of the 7 mice a few larvae of *A. duodenale* were found in the liver even when inoculations had been given by skin. This was true in only 1 instance with *A. caninum* and not at all with *N. americanus*. *N. americanus* on the other hand tended to be in the lungs especially at 6 days to a much greater extent than either of the other 2 species (Table III). In the intestine, *N. americanus* was not found at all, and the other species was present mostly at 6 days after oral or stomach inoculation.

Most pronounced among the 3 species were the differences in the persistence of the larvae in the tissues. In *A. caninum* even after 66 days 10% of the inoculated larvae could be recovered (Table I). In *A. duodenale* some larvae persisted in the host tissues for 66 days but in relatively low percentage after 12 days (Table II). In *N. americanus* on the other hand a few larvae were recovered from the liver at 24 days and from the muscles at 30 days, but even at 12 days only 5% or less could be recovered from all of the tissues collectively.

## DISCUSSION

It is evident that in the mouse the larvae of *A. caninum, A. duodenale* and *N. americanus* are able to enter the host via the buccal mucosa and lower levels of the alimentary tract as well as via the skin. It seems probable that this is true also in man. It is therefore reasonable to suppose that in those parts of the world where *Ascaris* and *Trichuris* infections are acquired from eating vegetables grown on contaminated soil or "freshened" with contaminated water, the infective stages of hookworm must to some extent similarly establish infections in individuals who have had no direct contact with contaminated soils. Smillie (1928) noted that under such circumstances orally acquired hookworm infections probably are not uncommon.

It has previously been shown that the larvae of *A. caninum* live for very long periods, up to a year or longer, in the tissues of abnormal hosts (Sawada, 1935, 1936; Nichols, 1956b). Schwartz and Alicata (1934) recovered *N. americanus* larvae from guinea pig tissues as late as 16 days after inoculation and Nichols found living larvae in mice up to 12 days but not at 15 or 17 days. Persistence up to 24 and 30 days has not previously been recorded. For *A. duodenale* no record has been found of persistance of larvae in experimental animals beyond the 66 days recorded here. As regards growth and development, Nichols found as did Schwartz and Alicata that the larvae of *N. americanus* undergo considerable size increase in the lungs of experimental animals, reaching the advanced 3rd stage in the lungs, whereas those of *A. caninum* show no size increase or development even after 392 days. It is not clear from the present or earlier experiments whether *A. duodenale* larvae entirely resemble *A. caninum* in this regard, but it is certain that they grow markedly less than *N. americanus* in the tissues of mice.

## SUMMARY

Mice were inoculated with infective-stage larvae of *Ancylostoma caninum, Ancylostoma duodenale* and *Necator americanus* via the skin, buccal mucosa and stomach. Using 500 larvae for each mouse, groups of 7 were inoculated with each of the 3 species and by each of the 3 methods. One mouse from each of the 9 groups was sacrificed at intervals up to 66 days after inoculation. Using a combination of peptic digestion and Baermann isolation from shredded tissues, larvae were recovered separately from the lungs, liver, small intestine tissues, small intestine contents and carcass (mostly muscles).

As many or more larvae of all species were recovered from the tissues after inoculation via buccal mucosa or stomach as via skin. In *A. caninum* most of the larvae promptly migrated into the muscles and about 10% of them persisted without growth for 66 days. The larvae of *A. duodenale* behaved similarly but they were also found in the viscera up to 36 days and their numbers were reduced more rap-

idly, reaching 1 to 2% of the inoculated number in 66 days. *N. americanus* larvae on the other hand underwent rapid growth, were found in the lungs and liver to a large extent, and disappeared rapidly from the tissues after 6 days, although 7 larvae were recovered from the liver of 1 mouse on the 24th day and 5 were found in the muscles of another on the 30th day. In the intestinal tissues and contents only the *Ancylostoma* species were found and those were in negligible numbers except at 6 days after buccal or stomach inoculation.

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## RESEARCH NOTE

### SOME HELMINTHS OF RACCOONS IN GEORGIA

Six raccoons of Central Georgia (Fort Valley and Dublin) were examined for endoparasites. Fourteen genera of helminths comprising about 16 species were recovered. They are as follows: (TREMATODA) *Apophallus venustus* (Ransom, 1920); *Euryhelmis squamula* (Rudolphi, 1819); *Eurytrema procyonis* Denton, 1942; *Fibricola texensis* Chandler, 1942; *Fibricola* sp.; *Pharyngostomoides procyonis* Harkema, 1942; (NEMATODA) *Arthrocephalus lotoris* (Schwartz, 1925); *Ascaris columnaris* Leidy, 1856; *Crenosoma* (?) *goblei* Dougherty, 1945 (single specimen); *Gnathostoma spinigerum* Owen, 1836; *G. procyonis* Chandler, 1942; *Molineus barbatus* Chandler, 1942; *Physoloptera rara* Hall and Wigdor, 1918; (ACANTHOCEPHALA) *Macracanthorhynchus ingens* (von Linstow, 1879); (CESTODA) *Mesocestoides lineatus* (Goeze, 1782); *Oorchoristica procyonis* Chandler, 1942.

Several of the helminths cited above have been incriminated by certain investigators as the direct or indirect cause of pathological lesions, which could prove fatal to the raccoon. The raccoon as a new host for *G. spinigerum* and the pathogenicity of the species to this host is to be reported elsewhere (paper in press).

Morphological variations within the genus *Euryhelmis* have been mentioned by several investigators, including Baer in 1931, Callot in 1946, and Senger and Macy in 1952. *E. monorchis* Ameel, 1938, described by Ameel (1938, J. Parasitol. **24:** 219–224), possibly is a degenerate form of *E. squamula*. Except for testes number (1), it shows close morphological agreement with the species. The male genital system in some specimens of *E. monorchis* examined by Ameel was entirely absent, probably an indication of progressing speciation. Specimens designated as *E. squamala*, from a North Carolina raccoon, were loaned by the U. S. National Museum. These trematodes were on a single slide (Helm. Col. No. 46460). Only a single testis could be discerned in 1 of the specimens, which also showed some variation in the disposition of vitellaria. A systematic study on variation shown by adult members of the genus is in progress and will be reported at a later date.—Bert B. Babero and Jacqueline R. Shepperson, *Department of Zoology, Fort Valley State College.*

# TRICHINIASIS IN DOGS AND CATS OF IOWA*

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Recent studies have been conducted to determine the incidence of trichiniasis in dogs and cats of Iowa. Garbage and wild animals, which are possible sources of trichiniasis in swine, may also cause infection in dogs and cats. Regulations recently enacted in most States prohibiting the feeding of raw garbage to swine may decrease the incidence of trichiniasis in wild as well as domesticated animals.

The prevalence of trichiniasis in dogs and cats of the U. S. has been checked primarily in city animals. Sawitz (1939) examined 300 dogs in New Orleans with only 1.3% positive, but found 10% of 90 cats positive. Cross and Allen (1948) found a much higher prevalence in dogs and cats in Chicago, since 16% of 100 dogs were positive as were 21.6% of 51 cats. De Giusti and Field (1952) found 4.5% of 180 dogs in Detroit to be positive and 1 of 20 cats. Riley (1928) reported finding *T. spiralis* larvae in 13.3% of 30 cats from St. Paul, Minnesota.

Zimmermann, Schwarte, and Biester (1956) reported only 1 of 2184 Iowa swine to be infected with trichinae. They compared the prevalence of trichiniasis in pork sausage from November, 1944 through June, 1946, with a more recent study, November, 1953 through June, 1955. In the earlier study the prevalence in bulk and link sausage was 12.4 and 11.7%, respectively, while in the 1953–55 series the figures were 1.2 and 3.4%, respectively. In addition, trichiniasis had been found in 6 species of Iowa wildlife, namely rats (11.8%), mink (14.1%), fox (11.9%) opossum (2.5%), raccoons (0.9%), and coyotes (50.0%).

## MATERIALS AND METHODS

Between March, 1954, and December, 1957, diaphragms of 521 dogs and diaphragms and tongues of 50 cats were obtained for examination for *T. spiralis*. All samples were obtained through the cooperation of the Department of Veterinary Pathology and the Iowa Veterinary Diagnostic Laboratory, Iowa State College. The animals were about equally distributed between rural and urban areas and included numerous breeds. About 20% of the animals were strays. The majority of the animals were young, although dogs up to the age of 16 years were included.

All samples were examined by the artificial digestion method, 45-gram (0.1 pound) samples being used when possible. When smaller samples were positive, results were expressed on the basis of a 45-g sample. The compression method was not used routinely because of its unreliability in detecting light infections.

## RESULTS

### DOGS

Samples from 521 dogs were examined during the period March, 1954 through December, 1957, 10.0% being positive. There was only slight variation in the prevalence during the 4 years of the study, the occurrences being: 1954, 11.6%; 1955, 8.6%; 1956, 11.4%; 1957, 8.0%. The infections found were generally light, the mean larval count per 45 gm for the 52 positive samples being 74.4. This is primarily a reflection of the infections found in only a few dogs since 44 of the

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52 positives contained less than 70 larvae per 45-gm sample. The maximum count of 930 larvae was found in a 5-year-old male dog of a Shepherd breed obtained from a rural area. Two other dogs, with counts of 728 and 429 *T. spiralis* larvae respectively, were obtained from a city dog pound.

A higher prevalence of trichiniasis was found in older dogs with a more or less gradual reduction with decreasing age. The prevalence for the various age groups was as follows: 13–16, 23.1%; 10–12, 32.4%; 7–9, 21.8%; 4–6, 12.0%; 3 or less, 1.9%. There were 121 dogs of unknown age examined, of which 7.4% were found to be infected.

Little difference in prevalence was noted between the sexes in the dogs examined. Of 209 known females, 9.6% were positive, while 11.2% of 242 males were positive. Seventy samples of unknown sex were also examined, with 7.1% positive.

There seems to be little difference between susceptibility of breeds of dogs to trichiniasis. At least 20 breeds of dogs were represented in the 52 positive samples.

### CATS

The number of cats included in this study was comparatively small but gives an indication of the incidence of trichiniasis in this animal species. Only 3 of the 50 cats examined were positive, the mean larvae counts for the three positive samples being 1174. The individual counts for the 3 positive samples were 60, 1106, and 2437, respectively. The cat with the highest count was a stray captured in a heavily wooded area where it had access to rodents and other possible sources of infection.

## DISCUSSION

Due to the moderate incidence of trichiniasis in dogs (10.0%) and cats (6.0%), it is interesting to contemplate the role that the dogs and cats may have in perpetuating trichiniasis in swine. Swine may acquire infections either by consuming infected meat or by fecal transmission from infected animals. It is unlikely that swine would have access to carcasses of infected dogs and cats, but Spindler (1953) obtained fecal transmission to swine from feces of infected dogs, cats, swine, and albino rats. Since many dogs, and to a lesser extent cats, roam in rural areas from farm to farm at night, often entering hog lots, its is possible that they may have a role in perpetuating trichiniasis in grain-fed swine.

The light intensity of most infections encountered, as shown by larval counts, suggests that the infections in most of the dogs were probably subclinical in nature.

The higher incidence of infection in older dogs follows the same general pattern found in other species as well as man. Wright, Jacobs and Walton (1944) reported similar findings for trichiniasis in man. They indicated that with a few exceptions there was a progressive increase in the incidence of infection with age. The number of possible exposures to trichiniasis experienced by the older dogs would have a definite influence on the rate of infection. The increased use of various prepared commercial dog foods and the decrease in accessibility of table scraps and garbage should further reduce the incidence of infection in dogs of all ages in the future. The decreasing incidence of trichinae in pork and pork products would materially reduce the potential infectivity of garbage and table scraps, thereby eliminating some possible reservoirs of infection. These influences undoubtedly reflect in the decreased prevalence of infection in the younger dogs.

There is little difference in the susceptibility of dogs by sex or breeds. Male dogs may have a tendency to roam more than females, so that the small differences between the sexes may be accounted for by this. At least 20 breeds of dogs were found infected by *T. spiralis*, indicating that there is little difference in susceptibility between the various breeds. Most of the positives, however, were in breeds utilized for hunting.

SUMMARY

During the period of March, 1954 through December, 1957, 521 dogs and 50 cats were examined for *Trichinella spiralis*. Ten percent of the dogs and 6% of the cats contained *T. spiralis* larvae. Infections, as indicated by larval counts, were generally light, although a few heavier infections were found. A definite increase in the prevalence of infection was found in the older groups of animals. There were no differences indicated in the occurrence of trichiniasis as far as sexes or breeds of dogs were concerned.

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## RESEARCH NOTE

### SIZE AND RATE OF DEVELOPMENT OF HOUSE FLY LARVAE IN ARTIFICIAL CULTURES CONTAINING PHENOTHIAZINE

Phenothiazine has been reported to affect the development of *Musca domestic* L. in artificial cultures (Kohls et al, 1957, Vet. Med. **52**: 108–110). It was reported that the drug was larvicidal in direct proportion to its concentration in the culture media. This present note summarizes differences in size and rate of development of house fly larvae observed in the above tests when conducted with phenothiazine N.F. in 2-micron particle size.

One hundred house fly larvae were removed from each of 3, two-day old cultures. Two of the cultures contained 1 and 2 g of phenothiazine N.F. in 2-micron particle size; the third culture was not treated. The average length of 100 larvae for the 1- and 2-g cultures was 5.047 mm and 4.012 mm respectively; for the control cultures the average length was 7.725 mm. These differences are highly significant at the 1% level. Therefore, these measurements indicate that phenothiazine N.F. in 2-micron particle size retards the rate of development of house fly larvae in artificial cultures since larvae from the untreated culture were 2.678 mm or 3.713 mm longer than larvae obtained from the 1- and 2-g-treated cultures. Adult house flies emerged in 20 treated cultures after 12 days, 2 days later than adult flies emerged from 10 control cultures.

Adult flies emerging from cultures treated with 4 g of 2-micron phenothiazine N.F. were appreciably smaller than adults from control cultures; no measurements were made. Reduction in size was not observed with ordinary phenothiazine N.F. until the level of the drug per culture was 20 g or above.—ROBERT E. KOHLS AND A. C. TODD, *Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin;* and R. J. DICKE, *Department of Entomology, University of Wisconsin, Madison, Wisconsin.*

# A PRELIMINARY NOTE ON THE OCCURRENCE OF *PULEX IRRITANS* L. AND *PULEX SIMULANS* BAKER IN NORTH AMERICA

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When in 1895 Baker described *Pulex simulans* he remarked that "Though distinct, yet it is very closely related to *P. irritans,* and might easily be confused with that species." *P. simulans* has been confused with *P. irritans* ever since. Baker himself (1904: 379) demoted *P. simulans* to a variety of *P. irritans;* subsequently Jordan and Rothschild (1908: 9) synonymized *P. simulans* with *P. irritans* without comment and the species has never been recognized again. But, as I point out below, *P. simulans* appears to be a good species and it is therefore deplorable that the differences between the two species concerned have not been elucidated before, for numerous records of *"P. irritans"* from North America must actually refer to *P. simulans,* and it may now be difficult in many instances to ascertain to which of the 2 species of *Pulex* the records refer. Likewise, work has been carried out in the U.S.A. on the vector-capacity of *'Pulex irritans'* in relation to plague; it seems beyond doubt that both *P. irritans* and *P. simulans* must have been used in these investigations, and much of the information obtained cannot now easily be assigned to either of the 2 species. The differences in host-associations between these 2 species of *Pulex* indicate that *P. simulans,* which is common on colony-forming rodents such as prairie-dogs, may play a far greater role in the preservation of natural reservoirs of plague than *P. irritans,* which occurs mainly on larger carnivores and man, and is therefore presumably of no importance in the transmission of the plague bacillus among wild rodents.

Here we have once again, as in the case of *Xenopsylla,* an excellent example of the great necessity for accurate taxonomic work, without which observations on the biology of possible vectors of disease lose most of their value.

It is essential that there should be a firm basis for our concept of *Pulex irritans* Linnaeus, 1758, for his description mentions both Europe and America and therefore may well have applied to a mixture of the European species and *P. simulans,* to say nothing of the fleas of other genera which his host-records and the illustrations he quotes show must have been included. His type-material, if he ever had any, is lost. I therefore designate as neotype of *Pulex irritans* Linnaeus, 1758, the male from which I drew the aedeagus (Fig. 2) and which bears the data "Budapest, Hungary, R. Kohaut, Coll. C. F. Baker"; the neotype is in the Rothschild collection of fleas in the Tring Museum. This specimen belongs to the only species of *Pulex* found in Europe, which is apparently also the only member of the genus which has become cosmopolitan.

*Pulex simulans* and *P. irritans* are apparently the only representatives of the subgenus *Pulex* in North America, but in Central and South America there are several forms (some of which are as yet undescribed) which are closely related to the 2 North American species, and I regret that I have no opportunity at present to deal with the whole complex of forms which make up the subgenus *Pulex.*

May I express the hope that authors who have recorded *Pulex irritans* from North America will re-study their material and publish notes stating whether any

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of their records refer to *Pulex simulans*. Otherwise it will be practically impossible to use a number of their records for future work.

## *Pulex simulans* Baker (species revocata)

*Pulex simulans* Baker, 1895, Canad. Ent. **27**: 65, 67.
*Pulex irritans* var. *dugesii* Baker, 1899, Ent. News **10**: 37.

Baker's description of *Pulex simulans* reads: "Mandibles [= laciniae] and hypopharynx [= epipharynx] very short, not reaching one-half length of anterior coxa; joints of labial palpi robust, first joints longer than second, third longer than 1 [obviously Baker mistook segments II–IV for I–III]; maxillary palpi, with second joint in female shorter than 4; anterior lobe of exsertible portion of penis [= aedeagus] (See Wagner, Horae Soc. Ent. Ross., T. XXIII, pl. X, fig. 25 k) with upper half as broad throughout as lower half just above base."

Baker's belief that the laciniae and epipharynx are as short in *P. simulans* as in *P. irritans* is incorrect; their length varies a great deal in both species, especially in *P. simulans*, but the range of variation is much the same in both species, and the mouthparts even tend to be rather longer on the average in *P. simulans* than in *P. irritans*, in which they do not usually reach beyond the middle of the fore coxa. It was this variability which led Baker (1899) to describe as *Pulex irritans* var. *dugesii* specimens of which the main characteristic is that the labial palp (which is about as long as the laciniae and epipharynx and is now more used for standard measurements) reaches to ¾ the length of the fore coxa or more, for syntypes of *P. irritans* var. *dugesii* in the Tring collection are specimens of *P. simulans* with labial palps which reach to ½ or at most to ¾ the length of the fore coxa.

The length of the 3 last segments of the labial palp is correctly given by Baker, but these lengths are similar in *P. irritans*.

Baker's statement that the 2nd segment of the maxillary palp is shorter than the 4th segment in the female is not confirmed by the available syntype (did he make a slip and write "shorter" instead of "longer"?) and in most specimens of both species the second maxillary segment is the longest of the 4.

The only importance of Baker's reference to Wagner's figure, which is of a species quite unrelated to *Pulex*, is to identify the structure to which Baker was referring, for the dorso-apical portion of the aedeagus in Wagner's figure shows such a strong superficial and fortuitous resemblance to the dorsal aedeagal sclerite in *Pulex* as to identify the latter structure beyond doubt as what Baker meant, and this structure is indeed more uniform in width in males which I identify as *P. simulans* (Fig. 1, d.a.s.) than in the neotype of *P. irritans* (Fig. 2, d.a.s.), which is from Baker's collection. There is some variation in both species, but, as shown in Figure 1, the dorsal aedeagal sclerite (which is a single structure situated above the aedeagal inner tube and therefore not to be interpreted as the aedeagal crochet) is broad throughout in *P. simulans*, whereas in *P. irritans* (Fig. 2) it is always relatively longer and far slenderer. Another important difference between the aedeagus of *P. simulans* and that of *P. irritans* is that in the former the small and elongate crochets (Fig. 1, cr.) are rodlike, while in *P. irritans* (Fig. 2, cr.) they are much expanded apically. There is of course some variation in these structures, also, for instance the expansion of the apical part of the crochet in *P. irritans* may be smaller than shown in the figure.

There seem also to be differences between the claspers of the 2 species, in *P. simulans* e.g. the processes $P^1$ and $P^2$ are shorter and $P^1$ often straight.

Apparently there are few characters which would distinguish the females of

*P. simulans* and *P. irritans* but the average size is much smaller in this sex of *P. simulans* and there are usually more setae (7–9, usually 4 or 5 in *P. irritans*) on each side of sternum VII.

The differences in size between the 2 species are: *P. simulans* ♂ 1.5–2 mm, ♀ 2–3 mm; *P. irritans* ♂ 2–2.5 mm, ♀ 2.5–3.5 mm.

Baker (1895: 67) mentions that "Two specimens of this flea, taken from opossum (*Didelphis virginiana*), were sent to me by Mr. L. O. Howard, from the U. S. Dept. of Agriculture collection." Thus no type-locality is given, but from a paper by Webster (1904) it becomes clear that the specimens of *P. simulans* were collected along Devil's River, Texas, where Webster observed a number of fleas in the pouch of an opossum which he had shot and sent specimens to the Dept. of Agriculture at Washington. Baker's assumption (1904) that *P. simulans,* now regarded by him as a variety of *P. irritans,* occurred accidentally on the opossum, is refuted by Webster, who states in his note that the section of Devil's River where he shot the opossum is not a "thickly settled one," and that, although he had attracted some fleas himself, "their abundance on the individual [opossum] from which they were taken, and the nature of the country inhabited by her, would lead me to look rather confidently for their recurrence on others of these animals, especially where opossums are, if anything, thicker than humans, and fleas *ad infinitum.*"

Although *P. simulans* seems to occur on a wide variety of mammals, it is perhaps primarily a parasite of prairie-dogs (*Cynomys*), but it is also commonly found on skunks (*Mephitis*), ground-squirrels (*Citellus*), opossums (*Didelphis marsupialis*), grey fox (*Urocyon*), coyote (*Canis latrans*) and deer (*Odocoileus*).

*P. simulans* has a wide range of distribution in the Americas, from the northwestern United States through Mexico and Central America to the northern half of South America.

Baker stated that he had 2 specimens of *P. simulans;* 1 female syntype is in the U. S. National Museum and 1 syntype, also a female, in the Rothschild collection of fleas at Tring. Since Baker described the male, he must have had more than 2 specimens, but Dr. Phyllis T. Johnson informs me that there are no male syntypes in the U. S. National Museum. Although females of *P. simulans* are not very diagnostic, it seems probable that any males which Baker had are lost; so in view of the fact that the syntype in the U. S. N. M. is very badly damaged I designate the female syntype in the Tring collection as lectotype of *Pulex simulans* Baker, 1895. The lectotype bears the following data: "on opossum, Webster, per C. F. Baker," to which I have added the locality Devil's River, Texas.

Excluding unmounted material and unaccompanied females, the following specimens in the Tring collection, recorded by Hopkins and Rothschild (1953: 114, 115) as *Pulex irritans,* are misdetermined specimens of *P. simulans:* Montana, Arizona, New Mexico, Texas (here also belongs the female syntype of *P. simulans* of which the locality was unknown in 1953), Mexico, Oregon (Reston and Green Mt.), British Honduras, Costa Rica and Cuba.

Through the kindness of Dr. Phyllis T. Johnson I have also seen the following specimens of *P. simulans* from the U. S. N. M. collection: Texas (opossum), Kansas (opossum) and Mexico (*Didelphis marsupialis, Cynomys mexicanus* and *Citellus variegatus*).

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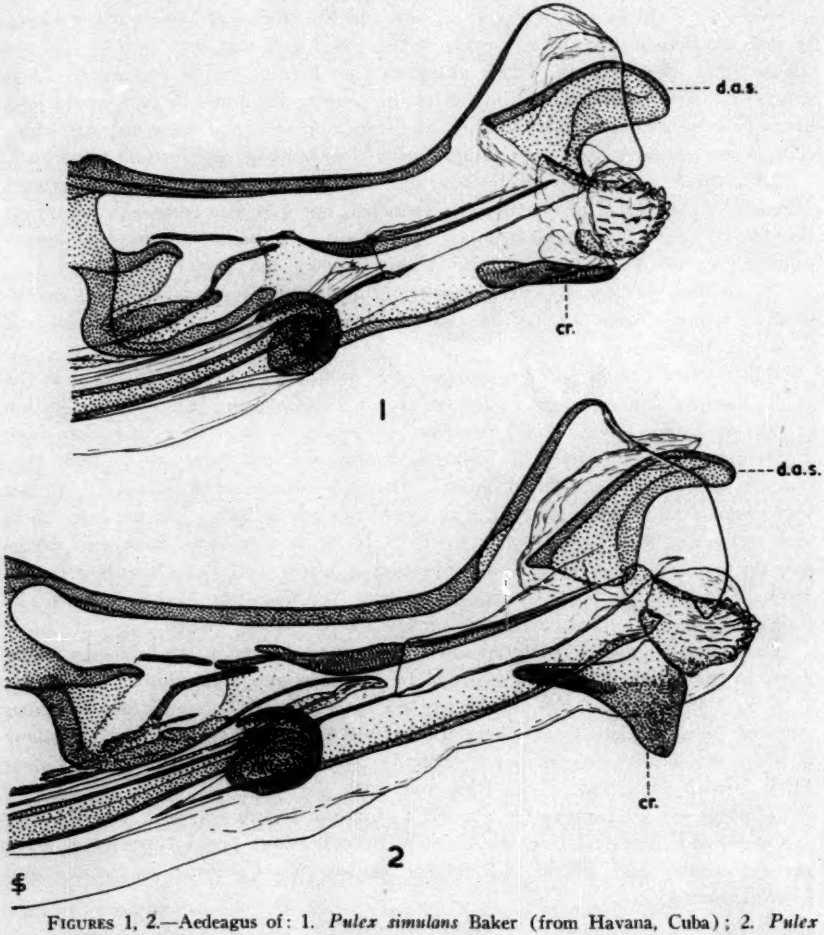


FIGURES 1, 2.—Aedeagus of: 1. *Pulex simulans* Baker (from Havana, Cuba); 2. *Pulex irritans* L. (neotype).

# ON *CUTEREBRA HORRIPILUM* CLARK (DIPTERA: CUTEREBRIDAE) PARASITIZING COTTONTAIL RABBITS IN WISCONSIN*

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Rabbit bots and other species of *Cuterebra* have received much study, but many facts remain unknown. Since bots were known to infest the Mearns cottontail rabbit, *Sylvilagus floridanus mearnsii* (Allen), in Wisconsin, the opportunity was taken to increase the knowledge of their morphology, biology and ecology. Besides detailed studies on *C. horripilum* Clark, an apparently rare species, *C. buccata,* was treated briefly.

## MATERIALS AND METHODS

Most of the rabbits were live-trapped in the University of Wisconsin Arboretum at Madison during 1955 and 1956. Small numbers were shot in other parts of the State in 1956. All live-trapped ones were tattooed and if infested were usually confined to an insectary for observation and for obtaining mature bots. After being studied the rabbits were usually released at their trapping site.

Bots in rabbits were detected by sight or by palpating. Indications of infestations were the swelling, cyst opening, protruding bot, shortness and scarcity of hair around the cyst opening and matting of hair with bot exudations. Immature bots were forcibly extracted by gripping and pulling the posterior segments with thumb and forefinger. Near-mature bots were induced to leave by killing their host or pinching the bottom of their cyst. Mature bots were collected after they dropped from their hosts. The throat region, the site of encystment of *Cuterebra horripilum* Clark, was skinned on dead rabbits in search of bots, exuviae and scars.

Eggs were dissected from a specimen of *C. horripilum* and counted. A free-hand drawing was made of the posterior spiracles of a first instar cuterebrid larva that was killed and preserved in 95% ethanol. All other drawings were made with the aid of a camera lucida. Second and third instar bots were preserved in 95% ethanol after immersion in K. A. A. D. (kerosene-ethanol-acetic acid-dioxane) for 8 to 24 hours, depending on the size of the larva. A puparium from which an adult *C. horripilum* emerged was cleared in 10% KOH at room temperature for 2 days. The posterior spiracular plates were then dissected as a unit and cleared in the KOH for 7 more days. After washing in water and 95% ethanol, the plates were placed on a slide and drawn as they began to dry. The genitalia were dissected from a pinned male *C. horripilum,* cleared in 10% KOH at room temperature for 48 hours and washed in water for 42 hours before immersion in glycerine.

Identification of *C. buccata* (F.) third instar bots was made by comparison with descriptions and illustrations by Knipling and Brody (1940). Third-instar *C. horripilum* larvae were identified by rearing to adults which were identified with the aid of Townsend's (1917) key and comments by Dalmat (1943b). Some adults were sent to C. W. Sabrosky for confirmation.

Mature and near-mature bots were confined to empty containers or to sand, soil or sod in efforts to induce and observe puparium formation. In 1955 the puparia were kept in 24×95-mm vials inside sealed chambers maintained at 24 to 36° C and approximately 93% relative humidity. The latter was maintained by a saturated $KNO_3$ solution according to Wexler and Brombacher (1951). The chambers were opened briefly at least once weekly to allow the oxygen content to return to normal. Mold was occasionally removed from some puparia.

All puparia were checked daily for adult emergence. Since the first 2 flies emerged too soon for insectary experiments, an attempt was made to delay further emergence. The puparia were held at 5 to 6° C and 96% r.h. for 1 hour the 1st day and 1 additional hour each of the next 3 days. After a fly emerged on the 4th day of chilling, the puparia were maintained at 5 to 6° C

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for approximately 3 months. All puparia were then weighed. The heavier ones were covered with 2 inches of insectary sand near a check puparium that had been there all winter. A small cage was placed over each puparium. Unusually light weight puparia were considered to contain dead insects and were discarded from rearing experiments.

When adults emerged in the insectary, observations on their behavior and efforts to stimulate copulation were made. The flies were allowed the freedom of the insectary, which was 7.5 feet high, 8 feet wide and 16 feet long, and were also confined to ½- or 1-cubic foot hardware cloth cages on sod. No water or food was supplied.

In 1956 all but 2 *C. horripilum* puparia were exposed for 1 month (November) to 5 to 6° C at 96% r.h. in an effort to obtain adults in less than 145 days. After exposure the puparia were buried just below the surface of moist sand maintained at 21 to 24° C.

## RESULTS AND DISCUSSION

### *Morphology*

*Egg.* Eggs of *C. horripilum* Clark are light brown and have the typical *Cuterebra* shape (Fig. 1), the usual tough, sculptured chorion, a cephalodorsal operculum (Fig. 2) and a broad ventral groove. The micropyle is at the cephalic end. The mean measurements of 20 specimens, in mm, are as follows: egg length, 1.43 (1.37–1.48); dorsoventral thickness, 0.32 (0.31–0.34); width, 0.29 (0.28–0.30) and operculum length, 0.38 (0.36–0.41) and width, 0.26 (0.26–0.27).

*First-instar larva.* One first-instar cuterebrid larva was collected (Figs. 3 and 4).

*Second-instar larva.* A few second-instar cuterebrid larvae were collected (Figs. 5 and 6). They are probably *C. horripilum*. Since specific determinations of first- and second-instar larvae are lacking, descriptions are omitted.

*Third-instar larva.* Of the third-instar *C. horripilum* bots that were reared to adults, the heaviest weighed 4.28 g and was approximately 18 mm wide and 38 mm long. In spite of the white integument, a mature or near-mature third-instar bot has a general black appearance due to the numerous large spines (Fig. 7). The larvae are subcylindrical and flattened on the ventral surface. Segment 8 is widest. The taper is gradual towards segment 1 and sharp towards segment 12. Segment 1, somewhat withdrawn into segment 2, bears the paired black mouth hooks. Dorsolaterad of each hook is a lobe with 2 sensory (?) papillae. The location of the internal anterior spiracles can be seen on the dorsal aspect near the base of segment 2. Segment 12 is truncate, typically withdrawn slightly into segment 11 and bears the subreniform, 3-part, rusty-red posterior spiracular plates (Fig. 8), the anus and the 2 lateral anal lobes. Eight shallow longitudinal channels apparently contain small, almost completely submerged spine bases. The 2 ventrolateral and 2 dorsolateral channels are well developed. The lateral pair and the pair midway between them and the dorsolaterals are not as distinct.

Spines are present on all 12 segments. Although several color patterns may be observed, most spines of mature larvae appear reddish-black except when light passes through the thinner parts which then appear dark red. In marked contrast, 3 to 5 rows of yellow-orange spines, with tips darker than bases, occur on the 4th 1/5th of segment 11. The posterior 5th of the segment is typically withdrawn anteriorly with segment 12 so that the posteriormost part of a larva is that bearing the yellow-orange spines. In detail, segment 1 bears on the ventral and lateral surfaces, but not the dorsal, approximately 4 rows of small white spines with pale yellow points. The approximate number of transverse spine rows on the tergum and sternum of segment 2 are 8 and 3; segment 3, 11 and 8; segment 4, 11 and 9; segments 5 to 11, 13 and 10 and segment 12, 4 and 5, respectively. The spines on seg-

ment 12 are small with the reddish-black dorsal ones graduating ventrally to yellowish-orange. Posteriorly directed spines occur as follows: all on segment 1, all on the anterior half and usually the adjacent row or 2 on the posterior half of segments 2 to 11, and the 2 short rows on the tergum of segment 12. Long, sharp, asymmetrical spines occur on the anterior and posterior of segments 2 to 11 and graduate with short, blunt, symmetrical ones in the median part of the segments. On each of segments 2 to 11, the spines posterior to the blunt ones are directed anteriorly. Except for the 2 short rows on the tergum, segment 12 has only sharp-pointed anteriorly directed spines. Rarely 1 of the reddish-black spines has 2 points, but all the others have only 1.

Small, pale yellow-orange sensory (Knipling and Brody, 1940) papillae are visible on segments 2 to 11. Four papillae are grouped close together in a transverse row at the anterior margin of the sternum of segment 2. Two rows of wider-spaced ones are located across the tergum. Along the middle of each of segments 3 to 10 is a ring of regularly and widely-spaced papillae; for example, segment 3 has 14 and segment 7 has 10 including a few double ones. Segment 11 has a row among both the yellowish-orange and anterior reddish-black spines.

A white *C. horripilum* third-instar larva, 10.5 mm wide by 20.0 mm long, which has only a faint rusty-red pigmentation of the points of its sharp spines has probably just cast its second-instar exuvium. The larger and presumably more mature larvae have more rusty-red pigmentation of the spines until the latter eventually appear reddish-black. The spines are also farther apart on the larger larvae. On this small third-instar larva, the largest spines measure up to 0.47 mm long on the longest side by 0.25 mm wide at the base compared with measurements of 0.67 mm and 0.57 mm, respectively, for spines of the mature larvae.

*Puparium.* All puparia were obtained by rearing. *C. horripilum* puparia have the typical *Cuterebra* appearance (Fig. 9). Larval segments 1 and 12 are invaginated so that the puparia have only 10 apparent segments. Farthest anterior and located on the anterior edge of the operculum are the 2 yellow, elliptical anterior spiracles. The subtriangular operculum (Fig. 10) is outlined by a low carina on the tergum of segments 1 to 3. The posterior spiracles are not visible although segment 12 may not be completely sealed off by segment 11. At first the integument is soft and reddish but becomes extremely hard and black. The reddish-black spines become black, and the yellowish-orange ones become dark red-orange. Segmentation is indistinct in normal puparia. A nonviable puparium formed by an immature bot was undersized, flattened, lacked external anterior spiracles and was distinctly segmented. A similar condition was reported in *C. emasculator* by Bennett (1955). Four puparia from which adult males emerged had the following mean approximate measurements: length 25.5 (25–26) mm; dorsoventral thickness, 14.4 (14–15) mm; and width, 15.0 (14–16) mm. Nine puparia from which females emerged averaged 26.9 (26–28.5) mm long by 15.8 (14–16) mm thick by 15.2 (15–17) mm wide. The difference between lengths was significant by the t test, but sample size was probably not large enough to give a reliable analysis.

*Adult.* Dalmat's (1943b) discussion of the adults of *C. horripulum* and *C. cuniculi* gives the key differentiating character: the first species lacks the large black shield on the mesonotum. C. W. Sabrosky confirmed 2 pairs of our reared adults as *C. horripilum* Clark.

The compound eyes of male *C. horripilum* adults are closer together than those

of the females. In a pair of apparently normal specimens, the narrowest width of the vertex between the compound eyes measured about 2.0 mm in the male and about 3.7 mm in the female. A more reliable sex determination without dissection may be obtained on the vertex width expressed in approximate median ocellar widths. These values for the male and female measured were 7.5 and 13.4 respectively.

When retracted the male genitalia of *C. horripilum* (Figs. 11 and 12) are in a genital pouch hidden by sternite 5. The surstyli and all but the setiferous posteriorly projecting lobe of each cercus are also hidden. Most of the terminology was used by Crampton (1942, 1944). Homologies are to be taken as tentative. The genitalia are described and drawn as if they were within the genital pouch.

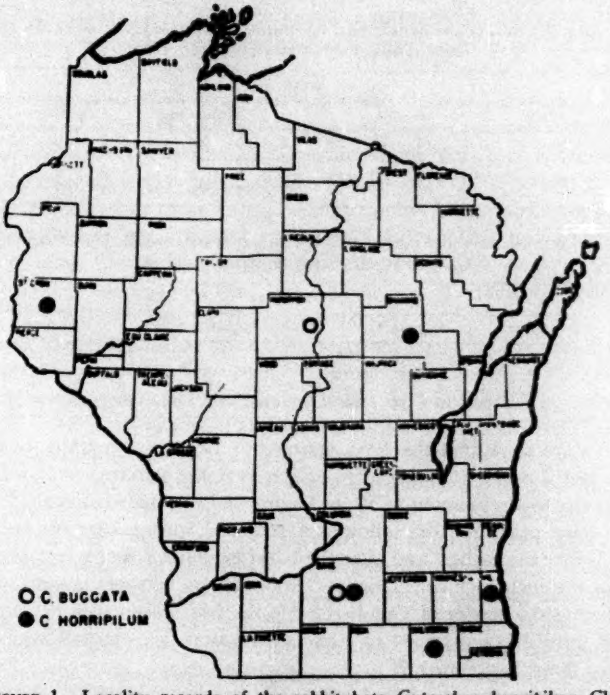
The prominent somewhat golf-club-shaped aedeagus has an elongate blunt epiphallus (Crampton, 1944) projecting ventrally at approximately a right angle. The distal part of the aedeagus is distinctly divided into lateral halves. The phallotreme is encircled by proximally directed titillators. The dorsal side near the base of the aedeagus is membranous. A median keel-shaped aedeagal apodeme extends dorsally. Its proximal end is pigmented least. Posterior to it is the small, subpyriform ejaculatory apodeme. From the sides of the base of the phallic structures, the paired, lightly pigmented, bladelike postgonites (Crampton, 1944) extend ventrally within the angle formed by the aedeagus and epiphallus. In lateral aspect, above and parallel to the aedeagus project the paired anteriorly directed toothlike lobes which seem to be part of sternite 9 (9S) as well as of the phallic parts and perhaps represent the pregonites of Crampton (1944). The dorsal margin of the hypandrium or sternite 9 is arched in dorsal aspect. Its 2 arms project ventrally so that in caudal aspect it has a horseshoelike appearance. The hypandrium arms attach first to the broader arms of the epandrium or tergite 9 (9T) and lastly to the long thin bacilliform sclerites of Crampton (1942) each of which is joined to a large surstylus. The surstyli appear to be appendages of the epandrium and as such cannot, as Crampton (1944) points out, be homologized with distimeres (harpagones) of lower Diptera as other investigators have done. Crampton (1942) also states that the bacilliform sclerites should not be homologized with the basimeres because they ". . . are probably merely secondarily developed sclerites which do not occur in all of the higher Diptera." Each surstylus has a thumblike mesocaudal process, each with a few large setae on its inner aspect. The inner aspect of the large ventral lobe of each surstylus bears a setiferous tubercle whose few setae are well developed. Very fine setae are scattered over the surstyli as revealed by setal membrane spots. The distal part of each ventral lobe is lightly pigmented. The cerci are subtriangular in the ectal aspect. The lateral corners are broadly rounded. A small rod projects from the median margin of each cercus towards the middle of the setiferous part of the opposite cercus. The ectal aspect of each cercus is moderately setiferous from near its center to the end of the posteriorly projecting lobe. A few of these long, bright yellow setae are shown in Figs. 11 and 12. The cerci are bordered along their lateral and ventral margins by large membranous areas except where lobes of tergite 10 (?) closely approach them. These lobes may be parts of tergite 9. The suture separating each from the adjacent integument may be the type in which chitin was secondarily reduced to give flexibility. Tergite 9 is approximately 2.0 mm wide.

*C. emasculator* male genitalia as illustrated by Bennett (1955) are similar to those of *C. horripilum* but show many differences: For example, no ejaculatory

apodeme is mentioned, and what would appear homologous to the lobes of 10 (?) T are mere rods.

### *Biology and Ecology*

Cottontails occur in all countries of Wisconsin (Wisconsin Conservation Department, 1935–1955). Two species of rabbit bots were collected: *Cuterebra horripilum* Clark and the comparatively rare *C. buccata* (F.). Locality records are too sparse to reveal much more than that both species occur in at least parts of the southern and central thirds of Wisconsin. Apparently rabbit bots have not been recorded from this state. *C. horripilum* was recorded from kittens by Dalmat (1943a). County records are shown in Text-Figure 1 and are listed below. For stable, precise locations the township, range and section numbers are included when available. Uncredited collections were made by the senior author.




TEXT-FIGURE 1. Locality records of the rabbit bots *Cuterebra horripilum* Clark and *C. buccata* (F.) in Wisconsin.

*C. horripilum:* Dane Co.: U. W. Campus, VI 18, 1954, ♀ indoors, R. J. Dicke, coll.; VIII 20–22, 1956, 4 larvae *ex* Mearns cottontails; U. W. Arboretum, VII 28– X 16, 1955 and VIII 4– X 13, 1956, numerous larvae *ex* Mearns cottontails. Milwaukee Co.: 3d instar larva in museum collection, no other data. St. Croix Co.: Twp. 29 Range 19 sec. 3, VII 27, 1956, 3rd instar larva *ex* Mearns cottontail. Shawano Co.: Gresham, IX 15, 1952, 3rd instar larva *ex* 4- to 5-week old kitten, C. J. Heagle, D.V.M., coll. Walworth Co.: Delavan, VI 30, 1940, ♀, Henry Dybas, coll.

*C. buccata:* Dane Co.: U. W. Arboretum, IX 12, 1955, 3rd instar larva *ex* Mearns cottontail. Marathon Co.: Twp. 28 Range 7 sec. 14, VIII 13, 1956, 3rd instar larva *ex* Mearns cottontail.

In determining the seasonal incidence of the bots, cottontails trapped more than 1 time were counted once in every week that they were taken but only once in the August and seasonal totals. All cottontails that showed evidence of previous parasitism were considered positive in an effort to counteract underestimation that resulted from undetected immature bots and the temporary and permanent removal of hosts from the trappable population. Observations on a few former hosts revealed that vacated cysts closed in approximately 2 days to form a scab which gradually broke off at its edges until it was gone within 10 to 14 days of bot departure. Nearly all bots were undetected until they had molted to the third instar. The single *C. buccata* bot collected in the arboretum was included in the bot incidence data for 1955.

In both years the seasonal incidence of *C. horripilum* bots in the arboretum (Tables I and II) was similar to that reported by Geis (1957) for southern Michi-

TABLE I.—*Bot infestation and visible past infestation of cottontails, University of Wisconsin Arboretum, July 28 to October 16, 1955.*

| Number of cottontails | Week of | | | | | | | | | | | | | Total individuals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July | | August | | | | September | | | | October | | | |
| | 24 | 31 | 7 | 14 | 21 | 28 | 4 | 11 | 18 | 25 | 2 | 9 | 16 | |
| Positive | 5 | 7 | 13 | 6 | 7 | 14 | 4 | 3 | 2 | 3 | 0 | 0 | 0 | 54 |
| Examined | 16 | 21 | 26 | 15 | 35 | 54 | 35 | 26 | 44 | 32 | 31 | 47 | 14 | 261 |

TABLE II.—*Bot infestation and visible past infestation of cottontails, University of Wisconsin Arboretum, August 4 to October 16, 1956.*

| Number of cottontails | Week of | | | | | | | | | | | | Total individuals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July | August | | | | September | | | | | October | | |
| | 29 | 5 | 12 | 19 | 26 | 2 | 9 | 16 | 23 | 30 | 7 | 14 | |
| Positive | 1 | 4 | 4 | 8 | 6 | 1 | 3 | 1 | 2 | 4 | 3 | 3 | 30 |
| Examined | 2 | 10 | 10 | 16 | 19 | 15 | 14 | 29 | 23 | 49 | 45 | 33 | 205 |

gan. The Tables show that the 1955 season was longer. No traps, however, were set on the last 3 nights of July 1956. Each year the percentage of infestation increased rapidly to a maximum of 50 in August and then slowly declined. The comparatively later peak of 1956 followed a retarded spring. Uninfested cottontails examined before the earliest and after the latest evidence of bot infestations were not included in the incidence calculations. Although no cottontails were tabulated as positive during the weeks of October 1955, the bot season was not considered as terminated until a bot dropped on October 16 from a cottontail which had been held captive since September 27.

The total percentage of infested cottontails was lower in 1956. A reason is that proportional to the rest of that season more animals were trapped during its last weeks. If only cottontails examined in August are considered, the bot incidence in 1956 was slightly higher with 37% of 46 cottontails infested as compared with 35.1% of 94 infested in 1955. Multiple infestations were also slightly higher in 1956. Eight hosts had 2 bots each and 3 carried 3 each as compared with the 1955 record of 9 cottontails with 2 bots each. Over an entire season some rabbits probably were host to more than 3 bots. Of 86 bot-infested cottontails in populations composed of approximately equal numbers of both sexes, 44 were males and 42 were females. Nearly all cottontails examined were juveniles.

Excluded from Table II were 2 immature third-instar *C. horripilum* bots that were found in a San Juan (?) rabbit shot in the arboretum on August 22, 1956. Although this rabbit had been seen at intervals for 3 months, it was not until August that its mandible appeared matted. Close examination revealed a bot in a putrid cyst on each side of the mandible.

The single *C. buccata larva* collected in the arboretum was found in a dermal cyst over the ribs on the left side of a cottontail. The specimen from Marathon County was in a dermal throat cyst as were all *C. horripilum* larvae.

According to Knipling and Brody (1940) past infestations of bots in rabbits were discovered by skinning and finding exuviae and scars. During this study the exuvium of a second-instar bot was found under the throat skin of a former host skinned approximately 3 months after parasitism. A dead late third-instar *C. horripilum* bot was found under the skin of a cottontail from the arboretum on December 12, 1956. Dorsocaudad of the right shoulder of the host, the bot, tightly enveloped in connective tissue, lay on its ventral side with its anterior end directed anteriorly.

One first-instar *Cuterebra* larva was collected. It was slowly swaying while standing erect on its organ of attachment on a tergite of a mid-segment of a nearly mature *C. buccata* bot. The latter had just wormed out of its cyst in the throat skin of a juvenile cottontail freshly killed in Marathon County. Whether these 2 bots were together in the cyst or came into contact in the throat fur is not known. More than 1 bot per cyst has been reported by Worth (1950). During this study 2 bots were found sharing the same cyst opening. Their cephalic ends were in separate cysts. Another bot was in a cyst with 2 openings. Perhaps cyst openings are occasionally used as entrances by first-instar larvae.

First-instar *Cuterebra* larvae have been reported standing erect on their organ of attachment (Parker and Wells, 1919; Ryckman and Lindt, 1954) and swaying back and forth as though awaiting a passing host (Parker and Wells, 1919; Ferris, 1920; Moilliet, 1950). It has been shown that by the time first-instar larvae have attained a length of 9 mm (Stunkard and Landers, 1956) or even 6.5 mm (Beachley and Bishopp, 1942) within their hosts and are apparently about to molt, they have no organ of attachment. Perhaps it is represented by the anal lobes.

The following observations on the host-parasite relation were made after the cyst wall and opening had been formed by the *C. horripilum* larvae. Pus and bloody fluids flowed through the cyst opening and matted the surrounding hair which occasionally sloughed off. A cyst opening that was approximately 1.0 mm in diameter when first detected was enlarged to approximately 16 mm by the enlarging bot which eventually protruded from the cyst. When part of segment 9 was visible, the bot was mature and wormed out by peristaltic movements. While hosts were in traps, 3 bots dropped between evening twilight and noon the next day. The time from entrance into the host to drop of the larva is probably 4 to 4.5 weeks.

After dropping, some *C. horripilum* bots began burrowing immediately. Others migrated and did not burrow unless they encountered loose soil. On concrete some crawled under debris where they formed puparia. Still others formed theirs in the open. Usually the puparium was formed within a day after a larva left its host, but the period varied to the third day, such as in the history of the first fly that emerged.

In the laboratory 1 bot took approximately 9 hours to excavate its puparium cell and form its puparium in a piece of slightly loosened sandy loam sod. The bot

loosened the soil with its mouth hooks, and peristaltic movements carried the soil particles out of the cell. In 3 hours the cell appeared large enough to accommodate the larva. Three and one-half hours later the bot worked its cephalic end up between its caudal end and the shallow side of the cell and then pulled its caudal end down the deep side. Thus, by folding against its venter, the bot turned around inside its cell in only 15 seconds. Now on its dorsum the bot wormed out of the cell so that the first 6 sternites were visible. In order to be on its venter, the larva spent the next 20 minutes slowly twisting to the left by contracting, expanding, and reaching out with its mouth hooks apparently to grip the sod for leverage. Thirty minutes later the anterior spiracles were noticeably evaginated, mouth hooks had disappeared, and the visible integument appeared like that of a puparium. Slight peristaltic movements progressed anteriorly. Finally, 90 minutes later quiescence was near. The entrance to the puparium cell was closed by the operculum and encircled by the excavated soil particles.

As shown in Text-Figure 2 the mean weights of female *C. horripilum* puparia were always higher than those of males, but the differences were not significant by t test. The greatest loss of weight occurred during the first 2 weeks after transformation from larvae. The weights were almost constant after the fifth week. Puparia were dry or slightly moist when formed. The surface moisture increased and reached a maximum at approximately the beginning of the second week. By the end of that week, the surface was dry.

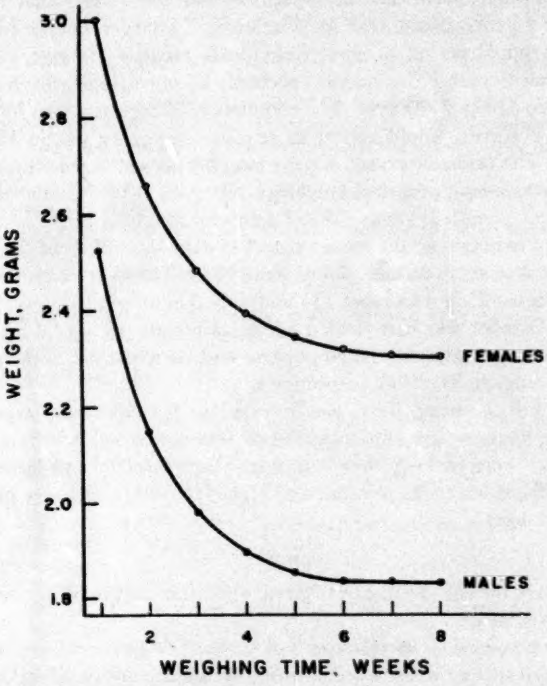
TABLE III—*Rearing histories of* Cuterebra horripilum *Clark collected as larvae in the University of Wisconsin Arboretum, 1955 and emerged as adults, 1956*

| Sex | Puparium formed | Adult emerged | Days in puparium | Dead |
|---|---|---|---|---|
| Female | Sept. 8 | Feb. 23–24 | 154–5 | Feb. 27 |
| Do | Sept. 12 | Feb. 24 | 145 | Do |
| Do | Aug. 13 | Feb. 28 | 199 | Feb. 28 |
| Male | Sept. 19 | July 2–3 | 287–8 | July 10 |
| Female | Sept. 8 | July 3 | 299 | Do |
| Do | Oct. 7 | July 5 | 272 | July 12 |
| Do | Sept. 11 | do | 298 | July 11 |
| Male | Sept. 7 | do | 294 | July 10 |
| Female | Sept. 7–8 | July 6–7 | 302–4 | July 11 |
| Male | Sept. 10 | do | 275–6 | Do |
| Female | Sept. 6? | July 8 | 309–11 | July 10 |
| Male | Aug. 9 | July 10–11 | 336–7 | July 12 |
| Female | Oct. 11–12 | July 11–12 | 273–5 | July 15 |

In Wisconsin *C. horripilum* adults probably emerge normally during the last half of June and possibly the first half of July following retarded growing seasons, such as in 1956. The outdoor-overwintered (check) puparium failed to yield an adult. The rearing histories of the 1955 arboretum bots are shown in Table III. The first 2 flies that emerged in the laboratory in February had not been cold-treated as pupae, but the third insect had been chilled during 4 days for a total of 10 hours. The others, all refrigerated for approximately 3 months, emerged in the insectary. Some abnormalities were an unretracted ptilinum of the first fly that emerged and unexpanded wings of the one that emerged, between 10:45 and 11:20 A.M., on July 8. The larva of the former probably had not attained full maturity. Of 21 apparently normal puparia from 1955, 8 failed to yield adults. The adult sex ratio was 4 males to 9 females or 44:100.

Adults were not observed feeding although both sexes were capable of everting and retracting their mouth parts. Bennett (1955) observed the drinking of water

by *C. emasculator*. The adults of *C. horripilum* sometimes attempted to escape from the small cages and frequently broke their wings and tarsi. When given more freedom they would usually walk upward before coming to rest. They were most commonly observed at rest, but cleaning movements were common, especially use of the prolegs on the head. If picked up or sometimes merely approached, both sexes made a low-pitched buzz and/or spurted a milky fluid out the anus. When released the few flies that were able would frequently take flight. Strong flyers of both sexes were not available at the same time. Copulation was not observed, possibly because it may take place only in flight. Ryckman and Lindt (1954) did not




TEXT-FIGURE 2. Decreases of mean weights of eight female and four male *C. horripilum* Clark puparia from which adults emerged.

describe flight or copulation but obtained larvae from eggs oviposited by a female *C. lepivora* after caging for 24 hours with a male in a 30″ × 17″ × 19″ cage before transfer to a pint jar. No adult *C. horripilum* survived more than 8 days. Some females occasionally protruded their ovipositor, but apparently none oviposited.

The egg capacity in *Cuterebra* is high. Dalmat (1943a) gave the capacity of *C. horripilum* as more than 1,000. Townsend (1915) estimated that *C. cuniculi* (*C. horripilum* ?) had 5,000 to 10,000 eggs. The abdomen of the female *C. horripilum* that was killed just after emergence contained only fatty matter. The specimen that lived 7 days had well developed eggs packed in its abdomen posterior to the paired 4.5-mm long air sacs. Apparently the eggs in this species are not developed at or before emergence as they are in *C. emasculator* (Bennett, 1955).

The second specimen contained 2,297 well developed eggs. This high capacity as Dalmat (1943a) and Townsend (1915) suggested is evidence for indirect oviposition. Cattle grub adults, *Hypoderma* spp., oviposit directly, and their egg capacities have been estimated as only 400 to 800 (Scharff, 1950). According to Townsend (1915) a species of *Dermatobia* that depends on other arthropods to carry its eggs to hosts has a capacity of less than 800 eggs. The egg capacity of *C. buccata* is apparently unknown, but in nature this species was observed to oviposit on a dead twig (Painter, 1930) and on the tips of blades of grass (Beamer, 1950).

Since the first bot to form its puparium (August 9), took the maximum time to emerge as an adult (336 or 337 days), it does not seem likely that *C. horripilum* has more than 1 generation a year in Wisconsin. A larger sample might have revealed a short pupal period in some individuals because Haugen (1942) used a cool, damp room to rear 1 *C. cuniculi* (probably *C. horripilum*) adult in only 61 to 63 days and 2 in 316 or 317 days. The completion of 2 generations by mid-October in Wisconsin, however, would appear to require emergence within 45 days by the second adult. The probable reason for the long bot season is that the tough chorion of the egg insures a long period of hatching. Gregson (1950) found that in British Columbia some *C. tenebrosa* eggs did not hatch for almost 1 year.

The attempt to obtain adults sooner than 145 days by chilling of the 1956 puparia for 1 month at 5 to 6° C failed. As of June 7, 1957 neither of two check puparia (formed on August 21 and October 15) and only 3 of 16 treated ones (formed from August 21 to October 14) had yielded adults: a female on May 1 or 2, a male on May 2 and a male on May 19. The puparia had been formed September 15, September 2 and August 30, 1956, respectively.

The last adult, a strong flyer, was released in the laboratory where it usually flew somewhat hoveringly to the windows or fluorescent lights. In a large lecture room the flights were in long sweeps at fairly high velocity and lasted less than a minute. The flight buzz was pleasant and higher in pitch and lower in volume than that of bumble bees.

## SUMMARY

Described are the egg, third-instar larva, puparium and male genitalia of *Cuterebra horripilum* Clark.

Numerous larvae of *C. horripilum*, 2 of *C. buccata* (F.) and one of an undetermined *Cuterebra* species were collected from Wisconsin cottontail rabbits, *Sylvilgus floridanus mearnsii* (Allen), most of which were trapped in the University of Wisconsin Arboretum. In the arboretum 54 of 261 cottontails were infested in 1955 as compared with 30 of 205 in 1956. An infestation peak of 50% was attained in August of both years. The earliest evidence of parasitism was detected on July 27 in St. Croix County, and the latest was noted on October 16 in the arboretum.

A *C. horripilum* larva excavated its puparium cell and formed its puparium in 9 hours. Weight losses of purparia containing living insects of this species were greatest the first 2 weeks after metamorphosis from the larvae. Nine female and 4 male flies were reared from these puparia in from 145 days at 24 to 30° C to 337 days after a 3-month delay at 5 to 6° C. One month at 5 to 6° C did not result in early emergence. A few flies were capable of sustained flight, but copulation, oviposition and feeding were not observed. All died within 8 days. A total of 2,297 eggs was dissected from 1 female.

ACKNOWLEDGMENTS

The authors wish to express appreciation to Dr. R. A. McCabe, Dr. R. S. Ellarson and Mr. J. R. Jacobson for assistance with the cottontail problems; to Mr. C. W. Sabrosky for taxonomic assistance and to all other persons who aided the study.

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## Explanation of Plates

Figure 1. Eggs of *Cuterebra horripilum* Clark. ×13.2.
Figure 2. Operculum of a *C. horripilum* egg. ×94.7.
Figure 3. Lateral aspect of a first instar *Cuterebra* sp. larva. ×70.1.
Figure 4. Posterior spiracles of the specimen in Figure 3.
Figure 5. Dorsolateral aspect of a second instar larva of a *Cuterebra* sp., probably *horripilum*. ×6.2.
Figure 6. Posterior spiracles of the specimen in Figure 5.
Figure 7. Ventral aspect of a mature third instar *C. horripilum* larva. ×2.7.
Figure 8. Posterior spiracular plates of a mature third instar *C. horripilum* larva dissected from a puparium from which a male fly emerged.
Figure 9. Lateral and dorsal aspects of *C. horripilum* puparia.
Figure 10. Ectal aspect of an operculum of a *C. horripilum* puparium. ×4.6.
Figure 11. Lateral aspect of the male genitalia of *C. horripilum*.
Figure 12. Ventral aspect of the male genitalia of *C. horripilum*.

PLATE I

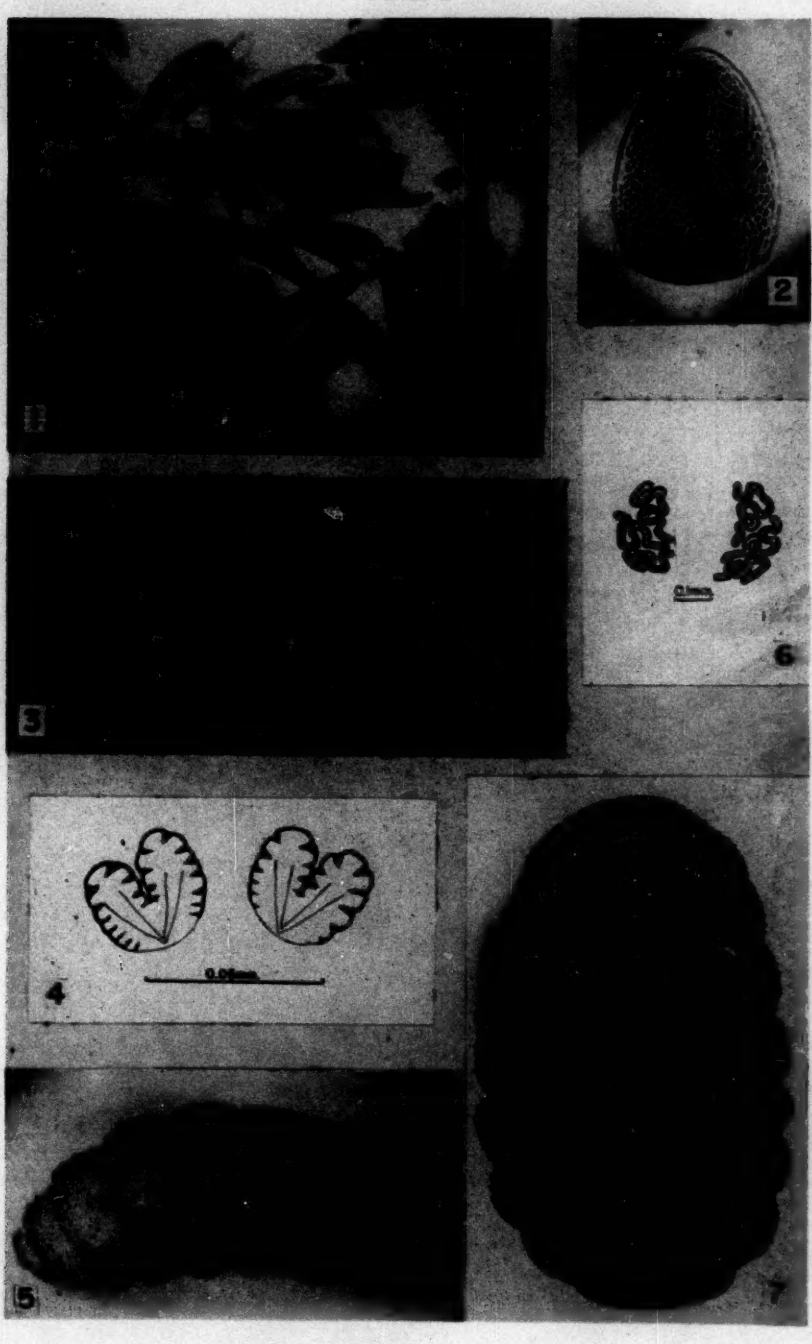
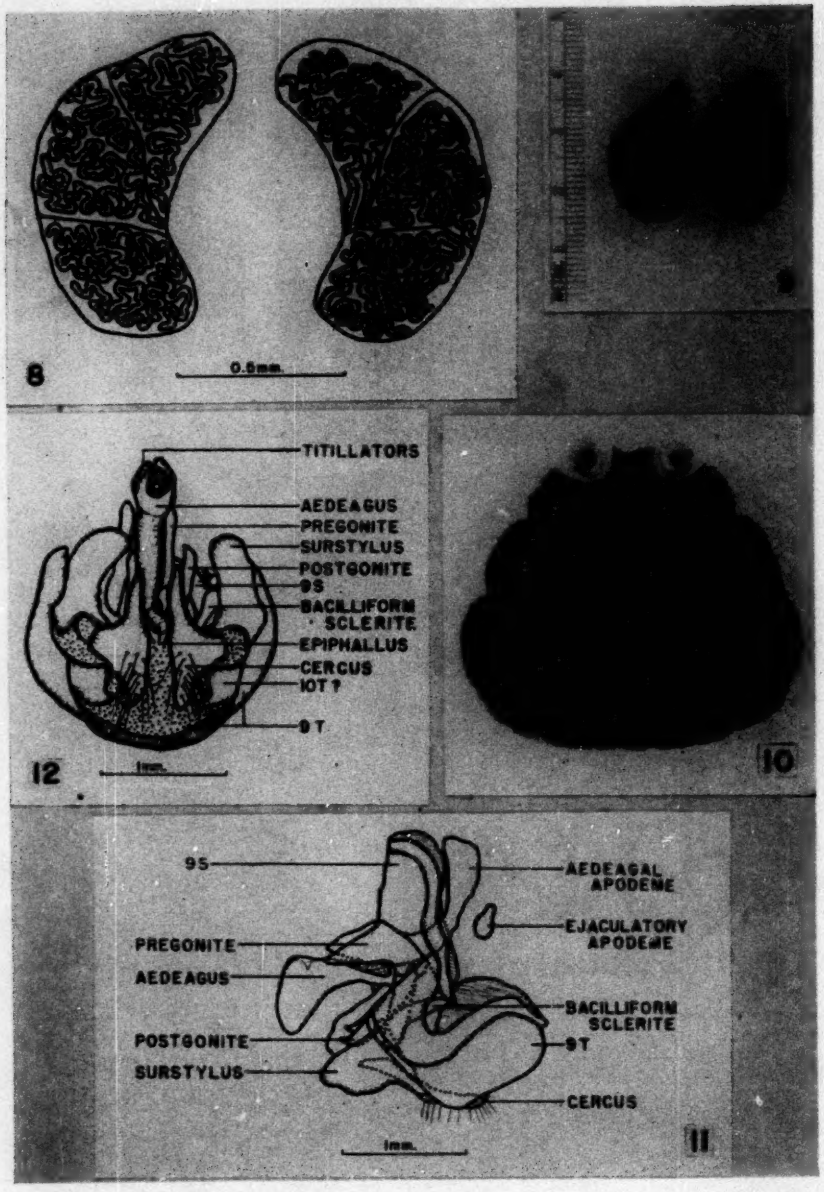


PLATE II


8
0.5mm.
TITILLATORS
AEDEAGUS
PREGONITE
SURSTYLUS
POSTGONITE
9S
BACILLIFORM SCLERITE
EPIPHALLUS
CERCUS
10T?
9T
12
1mm.
10
9S
AEDEAGAL APODEME
EJACULATORY APODEME
PREGONITE
AEDEAGUS
BACILLIFORM SCLERITE
POSTGONITE
9T
SURSTYLUS
CERCUS
1mm.
11

# *TUR UNISCUTATUS* (TURK) 1946 (ACARINA: LAELAPTIDAE) FROM NEOTROPICAL RODENTS

DEANE P. FURMAN[1] AND VERNON J. TIPTON[2]

The new genus and species *Protonyssus uniscutatus* was described by Turk (1946) from a single female specimen taken from the skin of a rodent, *Proechimys calidius calidius* Thos. captured in Northern Ecuador. Since *Protonyssus* Turk is a homonym of *Protonyssus* Trouessart, 1915, Baker and Wharton (1952) replaced it with the name *Tur*.

Recently the authors received collections of parasitic mites through the courtesy of Mr. Eustorgio Méndez and Captain Robert M. Altman. Among them were many mites from Panamanian *Proechimys* which bore a close resemblance to the description and figure given by Turk for *Tur uniscutatus*. Since Turk's description was based on a single specimen, mounted in balsam, it seemed possible that the differences noted from our specimens might be more apparent than real.

With the cooperation of Dr. Turk the type of *Protonyssus uniscutatus* was located at the Hope Department of Entomology, University Museum, Oxford, and made available for study through the kindness of M. W. R. de V. Graham. It was found to be identical with the Panamanian material, but due to its dorsal orientation and the type of mounting medium used, certain critical misinterpretations of structure were incorporated in the original description and figure by Turk (1946) causing him to place the genus in the family Macronyssidae. The description states that the digits of the chelicerae are devoid of all traces of teeth, whereas actually the fixed digit has a definite, although small, subterminal tooth. The "presternal" area of the sternal shield does not actually overlap the sternal plate as originally described, although superficially of that appearance in the type. The "very strong, slightly incurved line delimiting the genital shield from the ventral one" described by Turk is actually an internal sclerotization; the genital portion of the shield is not demarcated from the ventral portion. The anterior extremity of the genital area is slightly flared and fan-shaped, but is distinctly separated from the metasternal platelets.

The genus *Tur* is considered here as a typical member of the family Laelaptidae with close relationship to the genus *Laelaps*.

### *Tur* Baker and Wharton

*Female:* General facies of *Laelaps;* genito-ventral plate fused with anal plate or the separate plates in juxtaposition, the 2 regions bearing the usual 8 and 3 setae respectively. Sternal plate rectangular, broader than long, bearing 3 pairs of setae. Tritosternum bifurcate near basal segment with laciniae pilose. Parapodal plates bounding posterior margins of coxae IV but not fused with broad peritremalia, which extend anteriorly and dorsally over coxae I where they fuse with dorsal plate, and posteriorly nearly to caudal margins of coxae IV. Coxal spines heavy, expanded, stiff, except for that of coxa IV. Femora I and II with elongate setae at apices. Dorsal plate undivided. Tectum a rounded membranous lobe (indistinct in type). Chelicerae with elongate pseudosetae (indistinct in type) around base of movable digit, the latter lacking teeth; fixed digit dentate and bearing well developed pilus dentilis.

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*Male:* General facies of *Haemolaelaps*, but first pair of setae of holoventral plate extending almost to base of third pair of setae. Holoventral plate abruptly expanded behind fourth coxae, bearing 10 pairs of setae in addition to the 3 anal setae. Setae at apices of femora I and II slightly enlarged. Dorsal plate entire. Chelicerae with movable digit modified and enlarged as a spermatophore-carrier; the fixed digit elongate, delicate and bearing a seta; a row of pseudo-setae supporting a delicate membrane about base of movable digit.

Type of genus, *Protonyssus uniscutatus* Turk 1946.

The following redescription of the female of *Tur uniscutatus* is based on plesiotype material compared with the holotype. The male and nymphs are described for the first time.

*Tur uniscutatus* (Turk) 1946

Female (Figs. 1–5)

Idiosoma broadly egg-shaped, narrower anteriorly; 780 microns long, 570 microns wide; a moderately robust mite, strongly but not densely spinose.

*Dorsum*—(Fig. 1). Dorsal plate 692 microns long, 404 microns wide; antero-lateral margins heavily sclerotized and fused with peritremalia over coxae I; 39 pairs of setae measuring up to 115 microns long; a small pair between the long posterior marginal pair. Dorsal plate leaving uncovered wide lateral and posterior margins (width of margin varies depending on degree of engorgement) of dorsal cuticula, on which are about 10 pairs of robust setae. Tectum a membranous rounded lobe extending to mid-level of second movable segment of tarsus.

*Venter*—(Fig. 2). Laciniae of tritosternum pilose, extending to base of first movable segment of palps and divided to within a short distance of base. Sternal plate 83 microns long at mid-line; 174 microns wide at level of second pair of setae; widest posteriorly; posterior margin concave; bearing 3 pairs of robust, subequal setae measuring up to 98 microns long; 2 pairs of slit-like pores as illustrated; a third pair of pores on unarmed cuticula between sternal plate and metasternal platelets. Pair of metasternal setae similar to sternal plate setae, but 117 microns long. Genital, ventral and anal plates fused into a single plate 532 microns long, flared behind coxae IV to maximum width of 238 microns as illustrated (Fig. 2); lateral margins slightly concave anterior to anus, with acuminate caudal tip; bearing a pair of genital setae and 3 pairs of ventral setae 100 microns long, a pair of adanal setae 57 microns long and a postanal seta 82 microns long. A pair of small, elongate-oval, metapodal platelets behind coxae IV. Unarmed area of opisthosoma bearing 6 to 7 pairs of robust setae; posterior pair slightly fimbriate. Tubular peritremata extending anteriorly to level of anterior ¼th of coxa I; sinuous and turning dorsad over coxa I where broad peritremalia fuse with anterolateral margins of dorsal plate; peritremalia extending posterior of stigma almost to posterior level of coxae IV and bearing a small, subterminal pore. Parapodal plate almost encircling coxa IV, but free from peritremalia.

*Legs*—No unusual modifications. Legs IV 730 microns long to tip of caruncle, longer than I, and legs I longer than II and III. All with caruncles and weak claws. Coxa I with 2 stout, striated spines; coxa II with recurved anterior marginal spine and straight, stout, posterior spine, both striated; spines of coxa III similar to II but smaller; coxa IV with single normal seta. Femora I and II with 2 long, dorsal, apical spines. All legs moderately setose.

*Gnathosoma*—(Fig. 5). Deutosternal groove with 6 rows of teeth; 2 to 5 teeth per row. First movable segment of palp with ventral lobe, palpal genu with subterminal, raquet-shaped seta. Paired gnathosomal setae robust, striated, 47 microns long and 8 microns wide. Inner pair of hypostomal setae 60 microns long; outer pair 19 microns long; distal pair 37 microns long. Epipharynx with rounded apex and studded with short pseudospinules; Labrum a straight, pointed rod visible through the tectum. Chelicerae (Fig. 4) stout, 175 microns long; movable digit with recurved pointed apex, without teeth; fixed digit shorter than movable digit, with recurved pointed tip, 1 subapical pointed tooth, and a large, sickle-shaped seta arising from distal ¼th of digit; about 6 long pseudosetae arising around base of movable digit. Movable digit partially encased in a membranous sheath extending beyond apex of digit.

Numerous females each contained a single large, oval egg measuring up to 95 microns long and 67 microns wide.

Male (Figs. 6–8)

Idiosoma 550 microns long, 350 microns wide.

*Dorsum*—Dorsal plate 528 microns long, 323 microns wide; shape and setation similar to that of female, but setae more delicate, with small, posterior, submedian pair over ½ as long as large flanking pair. Uncovered cuticula narrow band bearing 4 or more pairs marginal setae.

*Venter*—(Fig. 6). Tritosternum as in female. Holoventral plate entire, coarsely reticulate, abruptly expanded behind fourth coxae, 432 microns long, 243 microns wide, bearing 10

pairs of setae in addition to usual 3 anal setae; first pair of setae extending almost to bases of third pair; 3 pairs of slit-like pores situated behind the first, second, and third pairs of setae respectively. Male genital opening on anterior margin between anterior-most setae of holoventral plate. Unarmed area of opisthosoma bearing 7 pairs of slender setae. Peritremalia broad as in female but turning dorsad over coxa II.

*Legs*—Similar to female but with coxal setae not inflated.

*Gnathosoma*—(Fig. 7). Unlike female gnathosoma in that paired gnathosomal setae not robust, but similar in size to inner and distal hypostomal setae. Tectum truncate, extending to middle of third palpal segment. Epipharynx as in female. Chelicerae (Fig. 8) 183 microns long; movable digit enlarged and modified as a spermatophore-carrier, the tip being recurved in cork-screw fashion in 1½ revolutions; fixed digit elongate, delicate, hyaline, extending beyond recurved end of movable digit, bearing a short seta proximal to its mid level and with a bifurcate tip; a row of short pseudosetae supporting a membrane about the base of the movable digit.

## Protonymph

Idiosoma 540 microns long, 183 microns wide.

*Dorsum*—(Fig. 9). Propodosomal plate coarsely reticulate; bluntly acuminate anteriorly, wider posteriorly, with truncate posterior margin at mid-level of coxae IV; 247 microns long, 165 microns wide, bearing 11 pairs of setae. Opisthosomal plate coarsely reticulate, smaller, rounded, 125 microns long, 175 microns wide, bearing 8 pairs setae. Three pairs small platelets located between propodosomal and opisthosomal plates. Unarmed dorsal cuticula bearing 10 pairs setae similar to those on dorsal plates, most setae minutely fimbriated.

*Venter*—Laciniae as in female. Ventral plate coarsely reticulate, 148 microns long, 104 microns wide, bluntly acuminate posteriorly, bearing 3 pairs of setae. Anal plate reticulate, broadly rounded anteriorly, abruptly acuminate posteriorly; anus located a distance slightly less than its own length behind the anterior border of the plate (Fig. 10); adanal and postanal setae as in female but smaller. A pair of small, ovate, metapodal platelets present. Unarmed ventral cuticula bearing 4 pairs of setae including a smaller pair between coxae IV. Peritremata proceeding dorsally at level of coxae IV and extending to mid-level of coxae III.

*Legs*—Similar to those of female but proportionately smaller, and coxal setae not enlarged nor spinose.

*Gnathosoma*—As in female except that gnathosomal setae not robust, but similar to inner hypostomal setae. Chelicerae as in female but shorter, and pseudosetae not visible at base of chelae.

## Deutonymph

Idiosoma 620 microns long, 405 microns wide.

*Dorsum*—Dorsal plate 553 microns long; 319 microns wide; oval shape; about 35 pairs of setae arranged similarly to those of female, although less numerous. Peritremalia in juxtaposition with dorsal plate at level of anterior margin of coxa I.

*Venter*—Tritosternum as in female. Ventral plate elongate oval, bluntly and broadly rounded anteriorly, widest between second and third pairs of setae, tapering posteriorly to a narrowly rounded tip at level of posterior margin of coxae IV (Fig. 12); 256 microns long, 131 microns wide; bearing 4 pairs of setae, all but the first located on lateral margins; a fifth pair on either side off the plate at the mid-level of coxae IV. Anal plate of inverted pear-shape with broadly rounded anterior margin reinforced with two to three concentric, sclerotized rings (Fig. 11). Post-anal seta approximately same size as setae of unsclerotized venter; adanal setae slightly smaller, but extending past base of post-anal seta. Tubular peritreme sinuous, extending from stigma between coxae III and IV to anterior ¼th of coxa I, turning dorsad over coxa I. Unarmed venter bearing about 7 pairs of setae, the posterior ones lightly fimbriated.

*Legs*—Similar to female but shorter and with coxal setae not modified into spines.

*Gnathosoma*—Similar to that of female but with gnathosomal setae not modified into spines, other setae proportionately smaller than in female. Chelicerae as in female but shorter.

Plesiotype female, male, deutonymph and protonymph deposited with the United States National Museum, Washington, D. C. Specimens from the same series as plesiotype female deposited with the University Museum, Oxford, England; Collection of the Department of Entomology and Parasitology, University of California, Berkeley, California; British Museum (Natural History), London, England; Muséum National d'Histoire Naturelle, Paris, France; Collection of Dr. Flavio da Fonseca, Instituto Butantan, São Paulo, Brazil.

Twenty females including the plesiotype were taken from *Proechimys cayennensis panamensis* Thomas at Cerro Azul, Province of Panama, Republic of Panama on September 22, 1955 by H. Van Horn. Two plesiotype males were established from a collection of 12 females and

6 males taken from 2 *Proechimys* at Madden Dam, Panama Canal Zone. The plesiotype deutonymph was taken with 55 females and 22 males from a single *Proechimys* at Madden Dam, Panama Canal Zone. The plesiotype protonymph was taken with 117 females and 1 male from *Proechimys* at Kilometer 10, Empire Road, Panama Canal Zone.

Many additional collections of *Tur uniscutatus* were identified from *Proechimys* of the Panama Canal Zone. A single collection of 14 females was recorded from *Didelphis marsupialis* of the same area.

According to Goldman (1920) *Proechimys* is very common and generally distributed in Panama. Of *Proechimys cayennensis panamensis* he states they are found as terrestrial inhabitants nearly everywhere except on the upper slopes of mountains. In some areas at least, *Proechimys* lives like a common rat in the huts and sheds of man.

An intriguing observation has been made on collections of mites from Venezuela, which may throw light on the phylogenetic relationship of *Tur* with *Laelaps*. Within a single collection of 5 mites from an unspecified host were found 2 distinct types designated "A" and "B", and 1 of intermediate type designated "A B". All mites of the series bear a close resemblance to *Tur uniscutatus* and are undoubtedly of the same genus.

Type "A", consisting of 1 female possesses a genito-ventral plate divided from the anal plate by a narrow space. Setae are stouter and longer than on *T. uniscutatus*, with a particularly spinose seta basally on trochanters I and II. Pilus dentilis of fixed digit is shorter and blunter than on *T. uniscutatus*.

Type "B", consisting of 2 females and 1 male, resembles *T. uniscutatus* more closely than does type "A". Female with genito-ventral-anal plate completely fused; coxal setae greatly inflated basally and with long, abruptly attenuate tips; gnathosomal setae similar to coxal setae; other setae, including inflated seta of fixed digit typical of *T. uniscutatus*. Male similar to *T. uniscutatus* but with coxal setae as in female of Type "B".

Type "A B", consisting of 1 female. Genito-ventral and anal plate, and inflated seta of fixed digit as in "A". Coxal and gnathosomal setae as in "B", but tip less attenuate. Trochanteral setae normal, as in "B".

The male of type "B", in common with *T. uniscutatus*, is readily differentiated from males of *Haemolaelaps* by the elongate first pair of holoventral setae, which extend well beyond the bases of the second pair of setae.

Males of the closely related genus *Eubrachylaelaps* possess a similar elongate pair of setae at the anterior of the holoventral plate, an indication of the close affinities of *Laelaps, Eubrachylaelaps* and *Tur*.

Type "A", referred to above, resembles very closely the figure and description of *Laelaps aragãoi* Fonseca 1938, (Fonseca, in litt., has pointed out that the original spelling, *aragonensis*, was in error.) with some minor differences, as in the size and shape of the basal trochanteral seta.

In light of the variation demonstrated in the degree of fusion of the genito-ventral plate with the anal plate in the genus *Tur*, Fonseca's species should be regarded as *Tur aragãoi*.

The authors acknowledge with appreciation the aid of Mr. E. P. Catts in preparing certain of the illustrations presented.

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## EXPLANATION OF PLATES

### PLATE I

*Tur uniscutatus*, female. FIG. 1. Dorsum; FIG. 2. Venter; FIG. 3. Dorsal view of tarsus I; FIG. 4. Tip of chelicera; FIG. 5. Ventral view of gnathosoma.

### PLATE II

*Tur uniscutatus*. FIG. 6. Venter of male; FIG. 7. Ventral view of male gnathosoma; FIG. 8. Ventral view of male chelicera; FIG. 9. Dorsum of protonymph; FIG. 10. Anal plate of protonymph; FIG. 11. Anal plate of deutonymph; FIG. 12. Ventral plate of deutonymph.

## RESEARCH NOTE

### ECTOPARASITES OF THE HOUSE SPARROW, *PASSER DOMESTICUS* (L.), IN KENTUCKY

Ectoparasites were collected from house sparrows, *Passer domesticus* (L.), trapped intermittently from August 22 through December 31, 1956, at Bardstown, Nelson Co., Ky. Seventy of the 77 birds were taken during August and September. The live-trapped birds were killed in polyethylene bags, both the birds and bags being examined for ectoparasites after 18 to 24 hours. In the following account, the numbers after the name of each ectoparasite represent, respectively, the number of birds infested, the number individually examined, percent infested, and total number of ectoparasites collected. Initially several birds were placed in a single bag and exact counts of some of their ectoparasites could not be made.

Acarina:

*Haemaphysalis leporis-palustris* (Packard) 5—75—7—7. The ticks detached quite readily, usually within 2 to 3 hours after the birds were killed. One nymph and 6 larvae were collected in August and September.

*Dermanyssus gallinae* (DeGeer) 2, *Ornithonyssus sylviarum* (Canestrini and Fanzago) 5, *Haemolaelaps casalis* (Berlese) 7. Individual infestation was not noted for these mites, 14 specimens being collected from 77 birds.

*Ptilonyssus nudus* Berlese and Trouessart 3—70—4—4. No special attempt was made to find nasal mites, the specimens collected having crawled from the nares.

*Dermoglyphus* sp. (near *elongatus* (Megnin)) 2—77—3—13. Feather quills were not examined internally for these mites, all specimens collected externally.

Mallophaga:

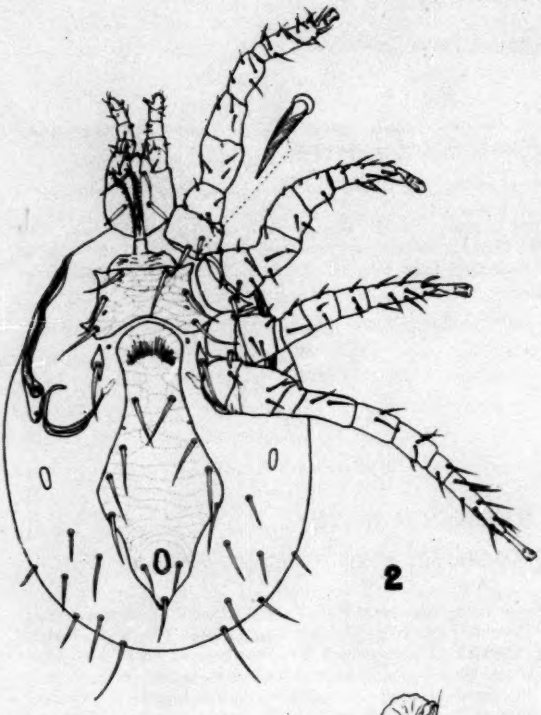
*Bručlia subtilis* (Nitzsch) 8—64—13—34. This was the most numerous ectoparasite.

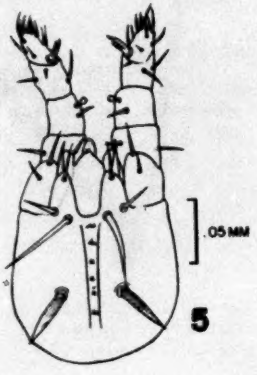
Diptera:

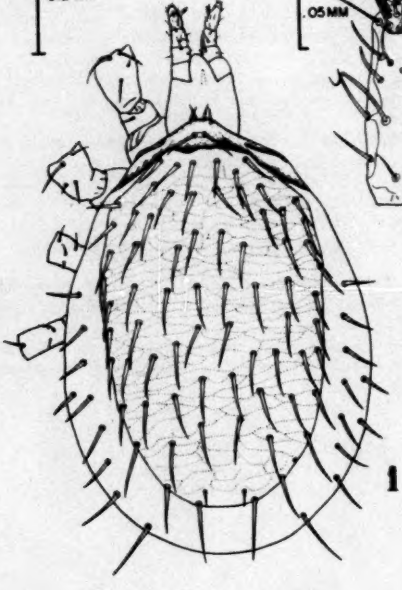
*Ornithoica vicina* (Walker) 3—77—4—3. Two additional hippoboscids, which appeared to be the same species, were seen to escape from the hosts during handling. The 3 specimens were collected 24 Aug., 2 Sept., and 12 Oct.

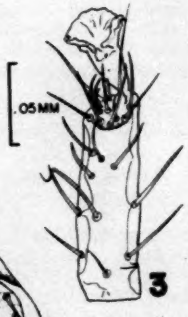
Acknowledgment is extended to R. W. Strandtmann, E. W. Baker, and Phyllis Johnson for identifying *Dermanyssus*, *Dermoglyphus*, and *Bručlia*, respectively.—NIXON WILSON, *Dept. of Entomology, Purdue University, Lafayette, Indiana.*

PLATE I





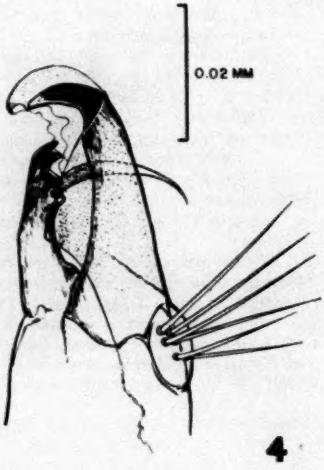
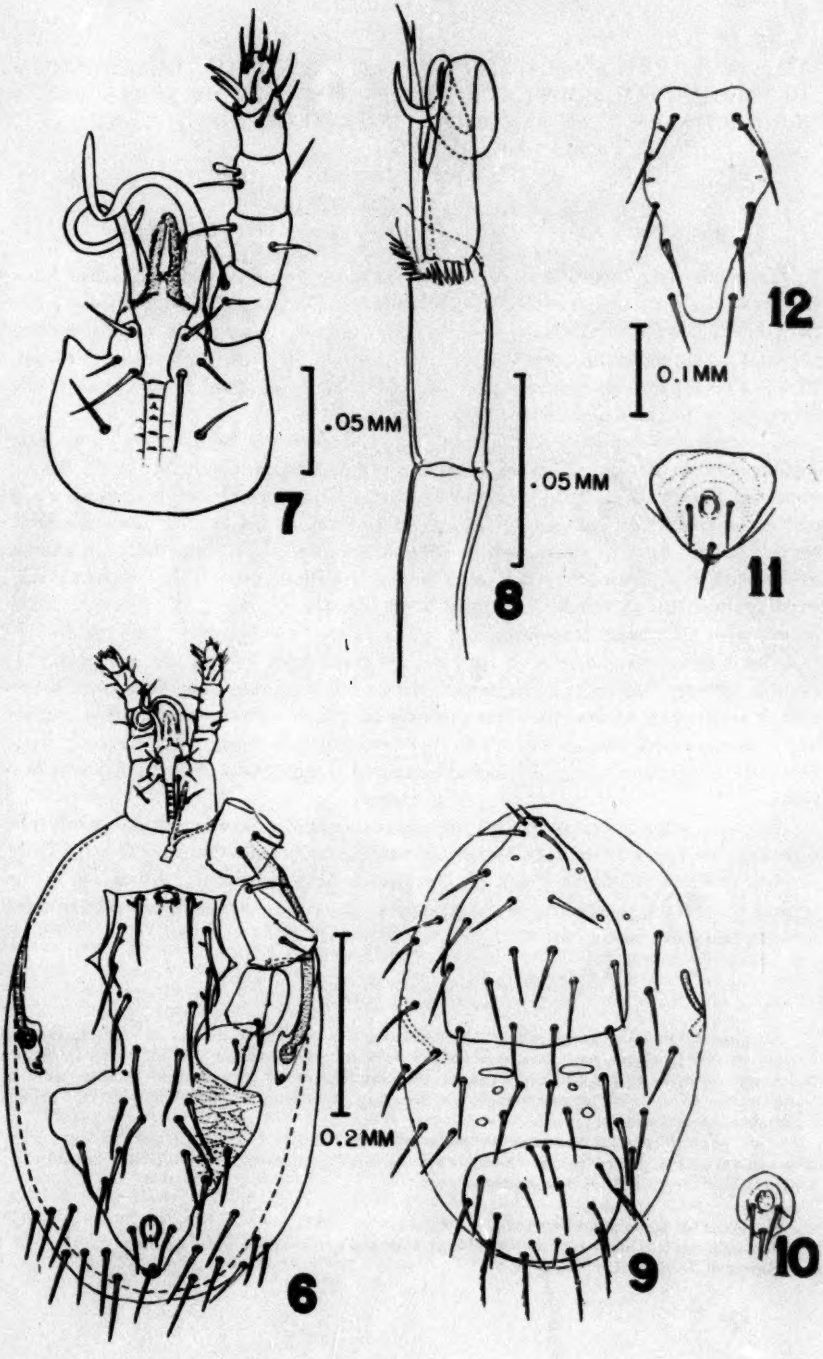



PLATE II



# NOTES ON AFRICAN *HAEMAPHYSALIS* TICKS. IV. DESCRIPTION OF EGYPTIAN POPULATIONS OF THE YELLOW DOG-TICK, *H. LEACHII LEACHII* (AUDOUIN, 1827) (IXODOIDEA, IXODIDAE)

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*Haemaphysalis* species and subspecies, although frequently distinguished from each other by only unspectacular combinations of characters, seldom display sufficient morphological variability to confuse their identity. Among the African haemaphysalid fauna, the commonest species, the Yellow Dog-Tick, *H. leachii* (Audouin, 1827), a vector of both human and canine diseases (Hoogstraal 1956), is a notable exception to the above generalization.

Contemporary workers feel certain that this species is represented by at least 2 subspecies in Africa. The second form, referred to provisionally as *H. leachii muhsami* Santos Dias, 1954, requires further morphological and biological study and taxonomic investigation to establish whether this is the earliest name available for this form. At any rate, numerous populations of *H. l. leachii* and *H. l. muhsami* are difficult to separate from each other and more definite criteria than those already existing need to be sought. A third form, *H. leachii indica* Warburton, 1910, occurs in the Oriental Region.

The original specimens of *H. leachii*, described from Egypt, are now lost (M. André, correspondence). Since large series from Egyptian populations are available, it is desirable to describe these carefully as a basic reference for further studies and to select a neotype (see below) to represent this subspecies. Concurrently, representative Egyptian material is being deposited in numerous important tick collections.

Eygptian populations of *H. leachii* are particularly valuable for this study not only because Egypt is the type locality of the species but also because this is one of the few areas in Africa in which *H. l. muhsami* does not occur. Variation in the typical subspecies, therefore, can be studied with assurance that the two subspecies are not being confused.

## *Haemaphysalis leachii leachii* (Audouin, 1827)
(The Yellow Dog-Tick)

Original description (page 185): "IX.9. *Ixodes Leachii*, Ixode de Leach. Les Ixodes ou *Cynorhaestes* d'Hermann sont remarquables par leurs palpes, engainant plus ou moins le suçoir, et formant avec lui un bec avancé et court. L'espèce figurée ici offre quelque analogie avec le *Cynorhaestes reduvius* d'Hermann; mais elle paroît en être distincte. 9.1. Individu trēs-grossi. l' grandeur naturelle."

*Note:* The illustration mentioned above appears in Savigny (1826) and portrays an Egyptian specimen; this figure is reproduced herein (Fig. 1). Contemporary workers consider *C. reduvius* Hermann, 1804, to be a *nomen nudum*.

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The opinions and assertions contained herein are the private ones of the author.
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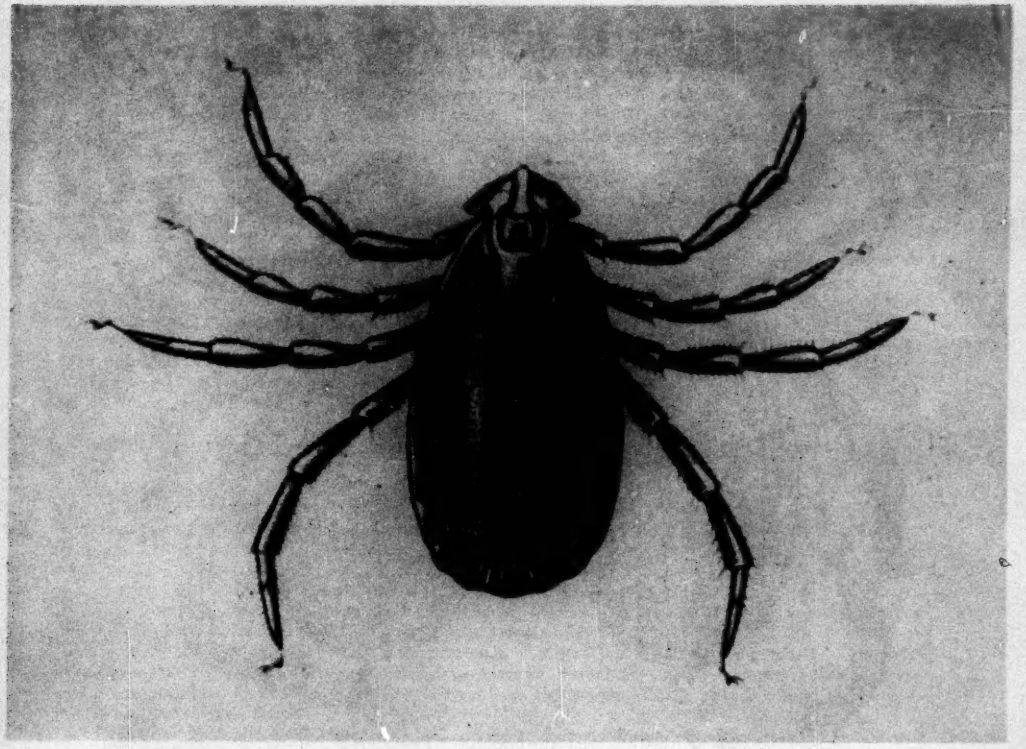


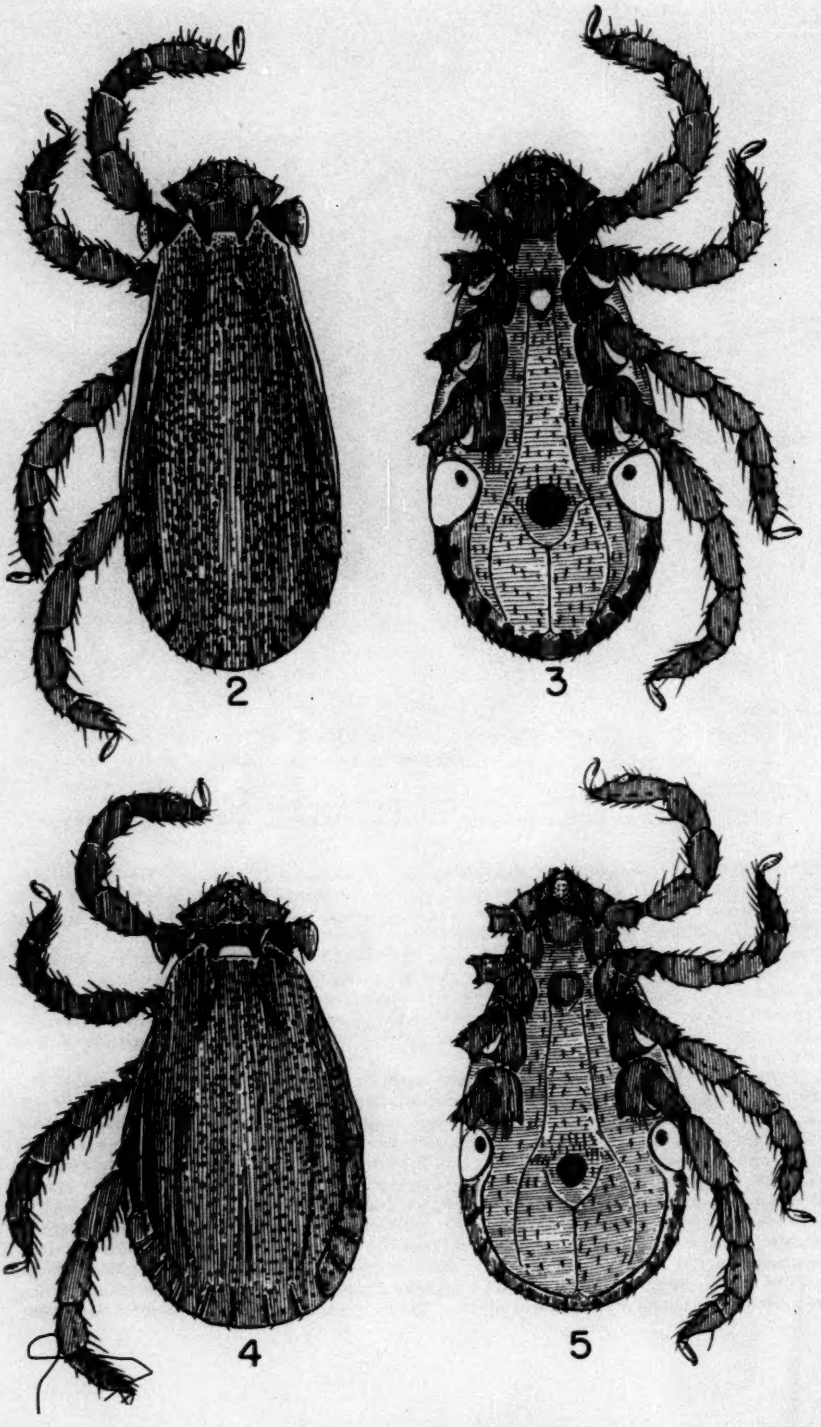
FIGURE 1.

*Redescription (of Egyptian populations)

* These descriptions are based on study of at least 200 specimens of each stage and sex. (Figs. 2 to 27)

**Male:** Typical specimens (narrow, elongate outline) (Figs. 2 and 3) measure (from apex of palpi to posterior margin of body) 1.95 mm to 2.80 mm in length and 0.96 mm to 1.20 mm in width. Less common (wide, short outline) (Figs. 4 and 5) measures 2.00 mm to 2.30 mm in length and 1.10 mm to 1.30 mm in width. *Body outline* (from apex of scapulae to posterior margin) narrowly elongate, length approximately twice or very slightly more than twice as long as wide; less commonly very slightly less than twice as long as wide (numerous specimens show gradation between these extremes); lateral margins very slightly to moderately convex (subparallel in extremely slender specimens), widest at or immediately anterior to level of spiracles; posterior margin gradually rounded; scapulae abruptly tapered and narrowly rounded apically in both forms. *Color* yellowish to reddish yellow.

*Capitulum* (Figs. 6, 7, 10 and 11). *Basis capituli* approximately twice as wide as long (including cornua) in wider specimens but width may (or may not) be reduced to 1.6 of length in narrower specimens; lateral margins slightly to moderately divergent anteriorly, posterior margin between cornua straight or almost so; cornua stout, triangular, comprising approximately 1/3 (rarely ½, also rarely ¼) total length in both forms and with apices more or less sharply pointed. Palpi obtusely angled and widely salient, widest at level of basal third; outer margin from apex to widest salience usually straight (in both forms), rarely very slightly convex or very slightly concave; sharply recurved from point of greatest salience in either a straight or slightly concave line to form a basal spur normally of acuteness and length illustrated (less commonly shorter and more obtuse, rarely somewhat more elongated); point of widest salience usually "down-tilted," rarely "up-tilted." Segment 2 (in both forms) from 2.0 to 2.5 as long as segment 3 (measured at point of greatest length of each segment); segment 3 with outer


2
3
4
5

margin approximately twice as long as inner margin. Palpal outline ventrally similar to dorsally except that basal spur is more variable (though only within limits illustrated, Figs. 7 and 11); segment 3 ventrally with a strong, comparatively narrow, elongate, tapering, retrograde spur with a narrow, rounded apex overlapping juncture of segments 2 and 3. Hairs as illustrated. *Hypostome* with slightly convex lateral margins and more or less bluntly rounded apex; length (from insertion of posthypostomal hairs to apex) from 2 to 2.5 of width; corona present; dental formula from 3.5/3.5 to 4.5/4.5 (rarely 5/5), files of 7 to 9 (rarely 10 or 11) denticles extending from corona to basal 5th of hypostome.

*Scutum*. *Outline* as noted above. *Lateral grooves* narrow, moderately deep (sometimes shallow), extending from anterior 5th of scutum to 2nd pair of festoons, sometimes continued as more shallow grooves to partially or entirely enclose 3rd pair of festoons. *Cervical grooves* sharply converging, of variable depth, thence extending as usually shallow, widely divergent grooves (often obsolete) to level of apex of lateral grooves. *Punctations* numerous, of moderate size, shallow but many slightly tipped and deeper anteriorly; on posterior half of scutum tending to be smaller, more regularly spaced, and more discrete; mostly non-contiguous; scattered over entire scutal surface including lateral ridges and festoons but usually absent from a narrow median area extending from scutal midlength to middle festoon and from a narrow line corresponding to the posterior margin of the female scutum; numerous punctations giving rise to a fine, white hair (these hairs usually partially or completely rubbed off). *Festoons* more or less clearly delimited (faint in many specimens).

*Legs*. *Coxae* with variable length-width ratio, each with a slight but distinct triangular spur that reaches or slightly overlaps the posterior margin at or immediately lateral of posterointernal juncture; these spurs somewhat variable within narrow limits but always distinct (frequently obscured by long hairs); each coxa also with a number of moderately long and very long fine yellowish hairs (hairs often entirely or partly missing due to rubbing during life or collecting); hairs on I and II numbering approximately 15 each, most of those on I more than ½ as long as coxa (i.e., very long), approximately ½ of those on II very long; hairs on III and IV numbering approximately 10 each, at least 4 of which are very long. *Trochanters* lacking ventral spurs; I dorsally with a large, triangular platelike spur flanked internally by 2 long hairs the outer of which is twice as long as the inner, also with 2 rows of hairs on anterior surface and several hairs on ventral surface. *Leg segments* with conspicuous ventral hairs, those opposite the spiracles especially stout and numbering 9 pairs. *Tarsi* I abruptly tapering, other tarsi gradually tapering; in shorter forms tapering margin sometimes slightly concave in lateral view; hairs as illustrated (Figs. 9 and 13). *Claws* long and strong. *Pads* (*pulvilli*) long, reaching to recurved apex of claws.

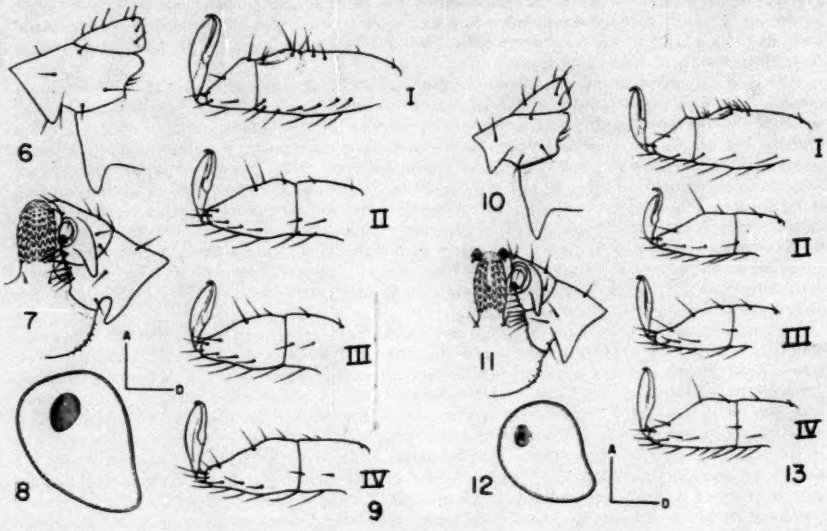
*Spiracular plates* equalling coxae IV in length; form as illustrated (Figs. 8 and 12). *Genital aperture* situated between coxae II; flap triangular, posteriorly with numerous hairlike projections. *Genital grooves* parallel or very slightly diverging from genital aperture to level between coxae IV and anus, thence widely curved to festoons. *Anal grooves* forming a pointed or rounded arch, either joining or not joining genital grooves; *anomedian groove* distinct, extending to middle festoon. *Anus* situated between spiracles; each valve with several fine hairs. *Festoons* ventrally usually more clearly marked than dorsally. *Hairs* ventrally in 7 or more irregular longitudinal lines extending full length of integument, somewhat more numerous in proximity of anus.

**Female** (Figs. 14 to 19): Closely corresponding to males in most non-secondary sexual characters. Differing from males as follows: *Size* larger, measuring (unengorged) 2.6 mm to 3.2 mm in length and 1.3 mm to 1.6 mm in width.

*Capitulum* (Figs. 16 and 17). *Basis capituli* also with lateral margins moderately diverging anteriorly; at least twice as wide as long, usually 2.4 as wide as long; with short, heavy, rounded or pointed cornua that are no longer than those illustrated and sometimes even shorter. *Porose areas* deep and distinct, varying from oval to subcircular, position as illustrated (Figs. 14 and 16). *Palpi* with dorsal outline as in male except that outer margin is more frequently mildly concave and the angle of greatest salience is more frequently very slightly upturned; basal spur dorsally usually more rounded apically than in male and not exceeding length illustrated. Ventrally, retrograde spur of segment 3 similar to but slightly larger than that of male; palpal outline similar to that of dorsal surface except (notably) for absence of basal spur; recurved basolateral margin joining basal margin as a curved line. *Hypostome* similar to that of male except for greater size and dentition, of 4/4, 4.5/4.5, or 5/5 (3.5/3.5 dental formula not observed).

*Scutum* approximately 1.25 as long as wide, rarely as little as 1.15 as long as wide; outline abruptly tapering posteriorly usually in a slightly sinuous line to fairly narrow apex, in some specimens tapering more gradually and as a straighter line to a somewhat less narrow apex (never bluntly rounded posteriorly). *Punctations* shallow, fairly small, usually numerous between outer margins and cervical grooves though frequently fewer than in specimen illustrated

(Fig. 14); few and widely scattered in central field though frequently more numerous than in specimen illustrated, in some specimens those in central field equalling lateral punctations in numbers and spacing; several lateral punctations give rise to a small white hair (these hairs




frequently rubbed off). *Cervical grooves* deep and converging on anterior 3rd of scutum, thence more shallow and slightly diverging to level slightly posterior of scutal midlength.

*Dorsal integument* with well marked lateral grooves and festoons, lateral grooves enclosing anterior 3 pairs of festoons; paramedian and posteromedian grooves usually well marked; 2 linear rows of small, pale hairs on lateral ridges, 2 to 4 others just inside lateral grooves, and 3 or 4 on each side of posteromedian grooves, also 3 or 4 hairs on each festoon.

*Legs* (Figs. 15 and 19) larger than but otherwise similar to those of male. *Spiracular plate* (Fig. 18) longer than coxa IV. *Genital aperture* large, U-shaped, situated level with basal half of coxae II. *Hairs* of ventral integument longer and somewhat more numerous than in males. Other characters as in males.

**Nymph** (Figs. 20 to 25): In general facies resembling females but differing from them in several salient features, especially in greater elongation of capitulum, absence of dorsobasal palpal spur, obsolescence of cornua, subcircular shape of scutum, and reduction of spurs on coxae IV and sometimes on other coxae. Body (from apex of palpi to posterior margin) measuring 1.40 to 1.60 mm in length and 0.72 to 0.90 mm in width (increasing to 3.2 mm in length when engorged).

*Capitulum. Basis capituli* approximately 2.5 as wide as long, lateral margins moderately diverging anteriorly, posterior margin straight or almost so; cornua reduced to mere slight points at laterobasal juncture. *Palpi* approximately twice as wide (combined width) as total length; point of greatest salience at basal 6th of length; outer margin straight or slightly concave (none observed to be convex); basal margin obtusely angled, lacking spur; segment 2 approximately 2.4 times as long as segment 3; outer margin of segment 3 twice as long as inner margin. Ventrally, palpal outline as from dorsal view except for production of basal margin into a long sublateral spur similar to but narrower than that of male; segment 3 with a narrow, tapering, retrograde spur slightly overlapping basal margin of segment 3. *Hypostome* similar to that of adults in shape, dentition 2/2 or 2.5/2.5 with 6 or 7 denticles in each file, corona obsolete or extremely weak.

*Scutum* subcircular, length and width approximately equal, margins rounded (as illustrated, Fig. 20) or very slightly more angular than illustrated; surface shagreened. *Cervical grooves* moderately shallow, slightly converging on anterior 4th of scutum, thence either obsolete or more shallow and extending to middle or posterior margin of scutum.

*Dorsal integument* similar to that of female. *Ventral integument* as illustrated (Fig. 21). *Spiracular plate* small, as illustrated (Fig. 24); length only slightly greater than ½ that of coxa

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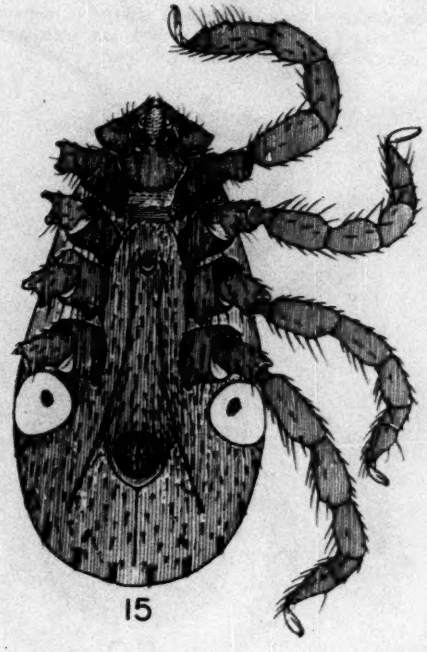

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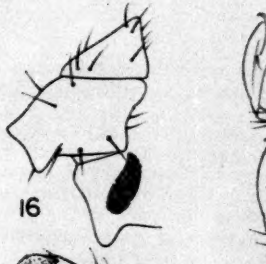

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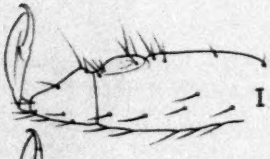


I

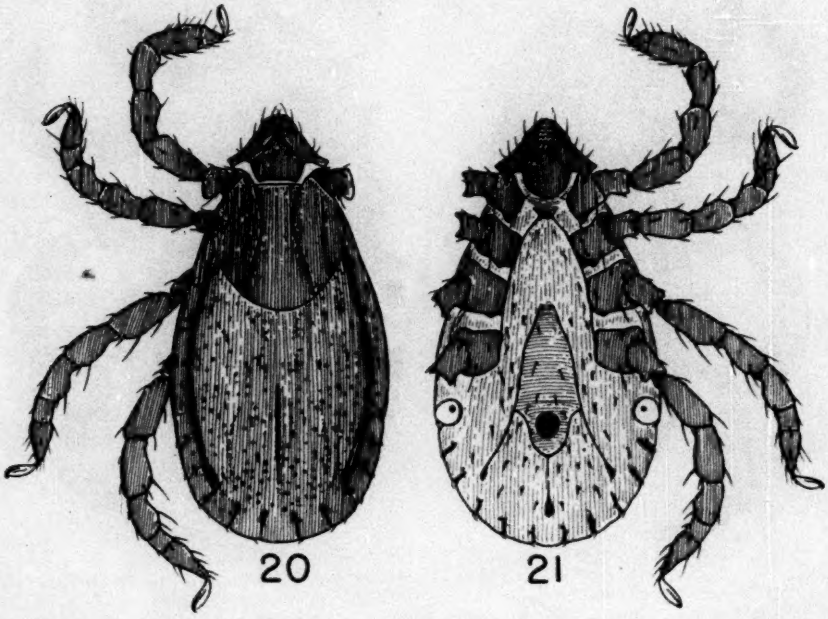
II

III

IV

19

IV. *Legs* similar to those of female except for reduction of coxal spurs, which on I to III may be similar to those of female or reduced to mere raised points on the coxal surface; spur on coxa IV even more reduced or obsolete; free segments lacking large numbers of strong ventral hairs, otherwise similar to those of females.




**Larva** (Figs. 26 and 27): Unengorged measuring approximately 0.60 mm in overall length and 0.45 mm at point of greatest width; scutum measuring approximately 0.21 mm in length and 0.34 mm in width.

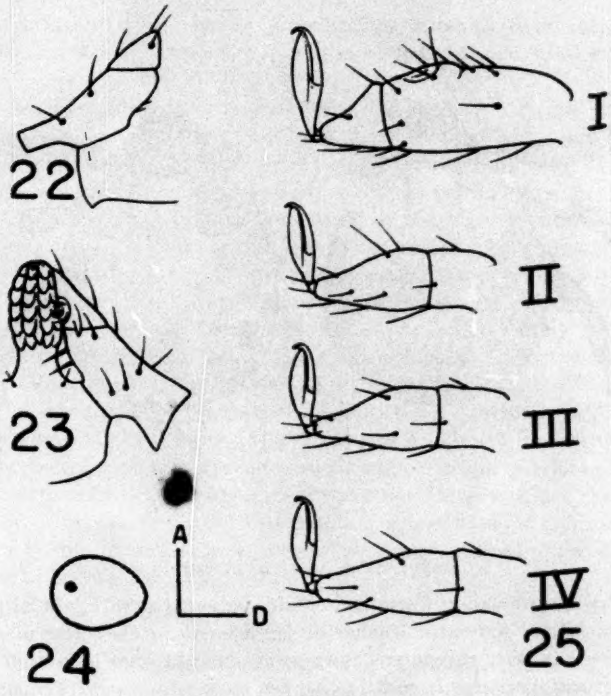
*Capitulum.* *Basis capituli* approximately 3 times as wide as long, lateral margins slightly diverging anteriorly, cornua faint or obsolete, posterior margin straight or slightly convex. *Palpi* widely expanded as in other stages; outer margin straight or almost so; basal margin dorsally produced as a large, wide spur, ventrally as a long, narrower spur; segment 2 somewhat longer than segment 3; segment 3 ventrally lacking retrograde spur. *Hypostome* 2½ times as long (from insertion of posthypostomal hairs to apex) as wide; apex rounded; dentition 2/2, with 5 or 6 denticles in each file; corona lacking.

*Scutum* transversely ovate; length (including scapulae) approximately ⅝ of width, widest at midlength, posterior margin gradually rounded from acute juncture with anterior margin; scapulae pointed; surface shagreened; cervical grooves parallel and distinct to approximately midlength, thence shallow and slightly diverging; 2 hairs in central field at level or midlength and 1 in each scapular field at level of anterior 4th of scutum.

*Dorsal integument* with complete, shallow lateral groove; 11 festoons each except midfestoon with a short hair; 3 hairs on each lateral ridge anterior of festoons, 2 hairs on central dorsum; posteromedian and paramedian grooves faintly marked. *Ventral integument* as illustrated (Fig. 27). *Coxae* lacking spurs though I is pointed at basal juncture of margins; each coxa with 2 hairs. *Tarsi* tapered. *Pad* (*pulvillus*) and claw of I large and stout, those of II and III smaller.

## Neotype

*H. leachii* was originally figured (Savigny, 1826) and described (Audouin, 1827) from Egypt, but the type specimen is now lost (Professor M. André, correspondence). A neotype (male unfed) has therefore been selected and deposited in the United States National Museum. This specimen bears the following data: Collected as an engorged nymph in nest of *Arvicanthis n. niloticus* (Desmarest, 1822) at Kirdasa, Imbaba, Giza, Egypt, 14 June 1953 by H. Hoogstraal (molted to adult in laboratory, 28 June 1953). Additional Egyptian males, females, nymphs and




reared larvae are deposited in the United States National Museum as well as in the collections of the following institutions and persons: Egyptian Entomological Society (ex Fouad I Entomological Society), British Museum (Natural History), Museum National d'Histoire Naturelle (Paris), Dr. J. Tendeiro (Lisbon), Musée Royal de Congo Belge (Belgium), Rocky Mountain Laboratory, Chicago Natural History Museum, Museum of Compartiave Zoology, Miss J. Walker (Muguga, Kenya), Dr. G. Theiler (Onderstepoort, South Africa), Dr. J. A. T. Santos Dias (Maputo, Mozambique), and the writer. Specimens will gladly be sent to other specialists upon request.

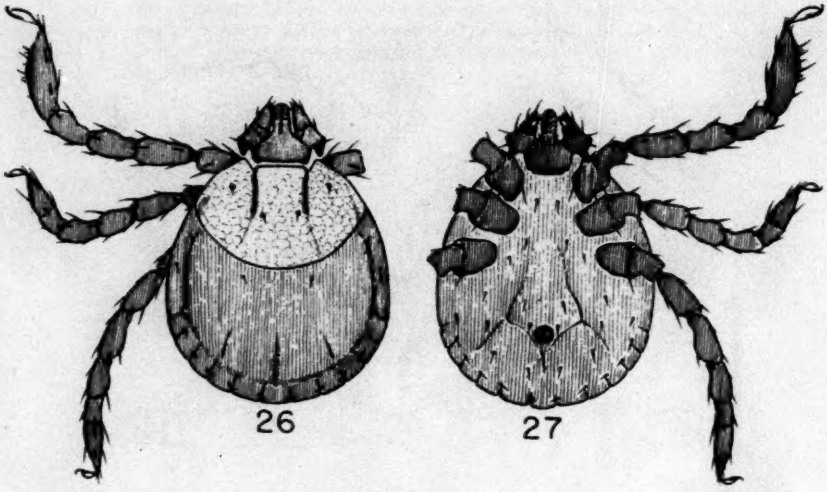
Approval for selection of this neotype has been obtained from Dr. Gertrud Theiler, Mr. Glen M. Kohls, and Miss Jane Walker. A copy of the present manuscript was sent to Dr. Francis Hemming, Secretary, International Commission on Zoological Nomenclature, before being submitted for publication.

## Asiatic Populations

Asiatic populations referrable to *H. l. leachii* that have been seen by the writer are the following:

Rocky Mountain Laboratory. Thailand: 3♂ ♂ from "tiger cat," Petchbari, Kowyog Nongchumpon, Nongpoe, 12 July 1952, Elbel. 1♂, 1♀ from "mongoose," Chaiyaphum, Poolhiew, Ban Nan Koom. *Assam:* 1♂ from "palm civet," Chabua, 10 January 1944. *Burma:* all from Myitkyina during various months of 1945. 158 adults and several larvae and nymphs from 5 *Viverra zibetha picta* (large Indian civet). 86 adults from 2 *Viverricula indica thai* (small Indian civet). 1♀ from *Panthera pardus* (leopard). 1♂ from *Panthera tigris* (tiger). 1♂ from *Gallus g. gallus* (forest fowl).

*British Museum (Natural History).* Several Asiatic lots are represented in these collections. Although my notes on this material are not available at the time this is written, they are worth mentioning owing to the previously moot status of *H. l. leachii* in Asia.




## DISCUSSION AND CONCLUSIONS

*Size and Morphological Variation.* Male specimens from Egyptian populations of *H. l. leachii* are somewhat smaller in size than those elsewhere in Africa. In Egypt, none has been encountered measuring over 3.0 mm in overall length; in tropical African specimens from 3.0 to 3.8 mm frequently occur. Females in Egypt are similarly smaller. The phenomenon of small size, undoubtedly due to the inclement local environment, is also true for other Egyptian populations of ticks, e.g. *Argas brumpti, Ornithodoros foleyi,* and *O. d. delanoei.*

Although Egyptian populations of *H. l. leachii* vary within a wider morphological range than is usually encountered in this genus, all individuals conform to the criteria previously proposed for this subspecies (Hoogstraal 1956) and none is suggestive of the closely related and sometimes intergrading subspecies, *H. leachii muhsami* Santos Dias, 1954, of tropical and southern Africa. It might be interpolated here that the question of taxonomic priority of the subspecific name *muhsami* requires further study. For establishing the typical form, range of morphological variability, and degree of intergradation between these 2 subspecies in Africa, Egyptian populations of *H. l. leachii* serve as a useful basic reference due to the absence of the second subspecies within this Republic.

The male characters in Egyptian populations (as well as in other African populations) that display an inordinate degree of variability for a form in this genus are: divergence of lateral margins and length-width ratio of basis capituli, size of cornua, lateral outline of palpi, degree of angle and extension of basal palpal spur dorsally and ventrally, apical bluntness of hypostome, dental formula, extension of lateral grooves to second or third pair of festoons, etc.

In exaggeratedly long and narrow specimens, i.e. the *"humerosoides* form" of Theiler (1943), there is frequently a tendency to more striking, large spurs and to more acute junctures of certain margins. Notably, however, this generalization

encounters an almost equal number of exceptions in which these junctures and spurs may be not merely "normal" but even more reduced than usual in acuteness and size.

*Biology in Egypt.* Immature stages of *H. l. leachii* parasitize chiefly grass-rats, *Arvicanthis n. niloticus* (Desmarest, 1822) in their nests, and less commonly hedgehogs, *Hemiechinus auritus aegyptius* (E. Geoffroy St. Hilaire, 1803), in their burrows. Grass-rats are numerous and widely spread in cultivated areas of the Nile Delta and Valley, an ecological situation shared by few other small mammals, e.g. commensal rats and mice, and shrews. Larvae and nymphs, both engorged and unengorged, as well as recently molted, unengorged adults, can be found in grass-rat nests at all times of the year. Adults parasitize the following carnivores: the jungle cat, *Felis chaus nilotica* de Winton, 1898; the common fox, *Vulpes v. aegyptiaca* (Sonnini, 1816); and the wolf jackal, *Canis aureus lupaster* Hemprich and Ehrenberg, 1833. We have not found this tick on domestic dogs or on the common commensal weasel, *Mustela nivalis subpalmata* Hemprich and Ehrenberg, 1833, in Cairo or in Nile Valley or Delta villages. More extensive search will probably reveal this tick on domestic dogs in areas where foxes, jackals, or jungle cats forage or where *Arvicanthis* rats occur. The infested carnivores noted above makes their dens at the fringe of cultivation or in the desert within a few miles of cultivation; they forage in the fields and on the desert edge. Carnivores confined to the desert have not been found bearing this tick.

ACKNOWLEDGMENTS

Dr. G. Theiler, Mr. Glen M. Kohls, and Miss Jane Walker have kindly consultated with the writer on this subject and have reviewed the manuscript.

SUMMARY

*Haemaphysalis l. leachii* (Audouin, 1827), originally described from Egypt, is redescribed and illustrated from Egyptian samples with special emphasis on morphological variation in local populations of this unusually versatile species. An Egyptian *neotype* specimen of this subspecies is deposited in the United States National Museum. The second African subspecies, *H. leachii muhsami* Santos Dias, 1954, often confused with the nominal subspecies in Africa, does not occur in Egypt. The present descriptions, therefore, are of added practical importance in establishing diagnostic criteria. Whether the name *muhsami* has taxonomic priority for the second subspecies requires further study.

In Egypt, larvae and nymphs of *H. l. leachii* feed chiefly on grass-rats (*Arvicanthis n. niloticus*) in their nests, and less commonly on hedgehogs (*Hemiechinus auritus aegyptius*) in their burrows. Nymphs molt to adults in these nests and burrows, both of which are found in cultivated, non-desert areas. Adults parasitize jungle cats (*Felis chaus nilotica*), common foxes (*Vulpes v. aegyptiaca*), and wolf jackals (*Canis aureus lupaster*) that forage in cultivated areas and make their dens at the fringe of cultivation or nearby in the desert.

Owing to the previously moot status of this subspecies in Asia, records of material referrable to *H. l. leachii* from Burma, Assam, and Thailand are furnished.

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### Explanation of Plates

Figure 1. Original illustration of species (from Savigny 1826, Plate IX, figure 9).

Figures 2 and 3, male elongate form, dorsal and ventral views.

Figures 4 and 5, male short form, dorsal and ventral views.

Figures 6 to 9, elongate form, capitulum (dorsal and ventral views), spiracular plate (A = anterior, D = dorsal), and tarsi I–IV.

Figures 10 to 13, short form, same as for 6 to 9.

Figures 14 and 15, female, dorsal and ventral views.

Figures 16 to 19, female, capitulum (dorsal and ventral views), spiracular plate (A = anterior, D = dorsal), and tarsi I to IV.

Figures 20 and 21, nymph, dorsal and ventral views.

Figures 22 to 25, capitulum (dorsal and ventral views), spiracular plate (A = anterior, D = dorsal) and tarsi I to IV.

Figures 26 and 27, larva, dorsal and ventral views.

## RESEARCH NOTE

### DISTRIBUTION OF PROTONEPHRIDIAL FLAME CELLS IN LARVAL *ECHINOCOCCUS GRANULOSUS*

Incidental to studies on host-parasite relations in echinococcosis currently in progress in this laboratory, we have made the following observations on the excretory system of *Echinococcus granulosus* which may relate to the metabolism of the parasite.

Protonephridial flame cells were seen in hydatid scolices of *E. granulosus* by several investigators (see Coutelin, 1931, Ann. Parasitol. **9**: 423–455). Although we have found them to be most apparent in vesicular scolices from in vitro cultures and in scolices which have evaginated, they may also be seen in normal invaginated scolices. In the latter, they occur near the periphery but are absent from the invaginated portion which contains the suckers and hooklets. The distribution of these cells is not consistent in hydatid tissues. For example, whereas Coutelin states that they never occur in the stalk which attaches the scolex to the brood capsule, we have seen them in this location on many occasions. In some cases they are completely within the stalk and in others they project into the lumen of the brood capsule itself.

Coutelin states, also, that flame cells were very rarely observed in the numerous brood capsule membranes he examined. In contrast, we have seen flame cells in some brood capsule membranes whenever we have searched for them. Most appear to be within or in close association with the excretory canals described by Coutelin. The brood capsules in which we have observed flame cells have been actively contractile and, in most instances, have contained not only fully formed scolices but also the solid germinal buds from which scolices evolve. We have not seen flame cells, however, in the germinal membranes of the few mother cysts we have examined carefully. Were this apparent difference between brood capsules and germinal membranes shown to be consistent, it could conceivably explain the difference in growth capacity of brood capsules and hydatid cysts proper. Accumulation of metabolic endproducts within the latter would result in continual imbibition of water from the host in response to an osmotic differential. (Aided by grants from the National Institute of Allergy and Infectious Diseases, U.S.P.H.S. (E-1769), and Pfizer International)—Calvin W. Schwabe and Lewis A. Schinazi, *Department of Tropical Health, American University of Beirut, Beirut, Lebanon.*

# ON A NEW AVIAN TREMATODE OF THE GENUS *CYCLOCOELUM* BRANDES, 1892

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The family Cyclocoelidae Kossack, 1911, includes all monostomate trematodes with intestinal ceca confluent, their hinder ends forming an arc. *Tamerlania* Skrjabin, 1924, is very close to the family *Cyclocoelidae* in having a similar type of intestine but differs remarkably in having the gonads much nearer the anterior end of the body. Therefore *Tamerlania* has been placed in a different family, *Eucotylidae* Skrjabin, 1924. A thorough study of morphology and life history of all the forms known under the family *Eucotylidae* would be worthwhile for determining the relationship between the two families.

There has been much controversy regarding the validity of different sub-families of the family *Cyclocoelidae*. Witenberg (1926), Lal (1939) and a few others accepted only 2 sub-families, viz. *Cyclocoelinae* Stossich, 1902 and *Typhlocoelinae* Harrah, 1922 as valid, whereas Joyeux and Baer (1927), Morishita (1924), Bhalerao (1935), Khan (1935) and a few others considered *Cyclocoelinae* to be the only valid sub-family. The 2 sub-families are differentiated mainly by the presence or absence of intestinal diverticula. Joyeux and Baer and their supporters considered the intestinal diverticula in *Typhlocoelum* to be only of generic importance, and as no other significant character separates the genus *Typhlocoelum* from *Cyclocoelum* it should not be considered of such significance as to separate the genera in 2 sub-families.

I strongly support this view and maintain *Cyclocoelinae* Stossich, 1902 to be the only valid sub-family of the family *Cyclocoelidae* Kossack, 1911.

There also has been much controversy regarding the validity of different genera known in the sub-family *Cyclocoelinae*. There were as many as 19 genera and several sub-genera known for this sub-family. These were reduced to 17 by Fuhrmann in 1928. Joyeux and Baer (1927) drastically reduced the number to 3 only: *Cyclocoelum* Brandes, 1892; *Typhlocoelum* Stossich, 1902 and *Spaniometra* Kossack, 1911. The remaining genera known for the sub-family *Cyclocoelinae* were considered by them to be synonymous with one or the other of the above 3 genera. Stunkard (1928) strongly criticized the common habit of many systematists of giving unnecessary importance to very minor characters, when he stated: "While the work (of Witenberg 1926) is a valuable contribution, the system in some respects is too arbitrary, too rigid, and ascribes too much importance to minor morphological features which in many cases cannot be more than individual variations." He has opined that life history studies are very important to establish the validity of new genera and new species. Krull (1940) has also realized the importance of life history studies to ascertain the correct relationship between different species and genera.

Lal (1939) studied all the species of the genus *Cyclocoelum* and separated them into 2 groups. Group I, with ovary distinctly pretesticular and not separated from the testes by uterine coils; Group II, with ovary distinctly between the testes and

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separated from them by uterine coils. He accepted all 3 genera mentioned above and considered *Haematotrephus* Stossich, 1902, to be a valid genus for the forms in group I. Thus according to Lal (1939) there are 4 valid genera in the sub-family *Cyclocoelinae*.

He gave the following key for the separation of the 4 valid genera of the sub-family *Cyclocoelinae*.

1. Intestinal ceca with diverticula ..... *Typhlocoelum* Stossich, 1902.
   Intestinal ceca lacking diverticula ..... (2).
2. Ovary distinctly pretesticular and not separated from the testes by uterine coils *Haematotrephus* Stossich, 1902.
   Ovary distinctly between the 2 testes and separated from them by uterine coils *Cyclocoelum* Brandes, 1892.
   Ovary distinctly posterior to testes and separated from them by uterine coils *Spaniometra* Kossack, 1911.

It seems that Lal mentioned nothing about *Typhocoelum* which may also have forms with the above 3 characteristic dispositions of the gonads, warranting the formation of 3 more genera.

Morishita (1924) divided *Cyclocoelum* species into 3 sub-groups: 1. *Mutabile type:* ovary between the testes and 3 gonads forming 3 points of a triangle; 2. *Vagum type:* ovary between the testes and all the 3 gonads lying in a straight line; 3. *Taxorchis type:* ovary in front of the testes and 3 gonads forming the 3 points of a triangle.

It seems that Morishita, like Lal, also failed to mention *Typhlocoelum* which may also have forms with the above dispositions of gonads as described by Morishita.

In view of the close resemblance between *Cyclocoelum* and *Typhlocoelum*, the different disposition of the ovary in relation to the testes, viz. slightly anterior or posterior or between them, should not be considered a generic character. I feel that these characters can be considered as group characters or at best characters of sub-generic importance. In light of the above discussion I disagree with the views of Joyeux and Baer and of Lal, and consider *Cyclocoelum* and *Typhlocoelum* to be the only 2 valid genera of the sub-family *Cyclocoelinae*. The other 2 genera, viz. *Haematotrephus* Stossich, 1902, and *Spaniometra* Kossack, 1911, are considered to be synonyms of *Cyclocoelum*.

With a slight modification of the views of Lal and Morishita I shall divide each of the 2 valid genera into 3 distinct groups:

I—Ovary distinctly pretesticular.
II—Ovary distinctly post-testicular.
III—Ovary distinctly between the testes.

Each of the 3 groups are further subdivided into 2 sub-groups:

A.—Gonads arranged to form 3 points of a triangle.
B.—Gonads arranged always in a straight line.

The division of 3 groups and 2 sub-groups as described above is also delineated in the diagrammatic representation in Figure 1.

Joyeux and Baer (1927), while reducing the number of genera recognized for the sub-family *Cyclocoelinae*, have retained *Spaniometra* Kossack, 1911 as one of the valid genera. On the contrary, I consider the genus *Opthalmophagus* Stossich, 1902, to be well defined and described much earlier than *Spaniometra*. *Opthalmophagus* should have been given priority over *Spaniometra*. *Spaniometra* therefore falls as a synonym to *Opthalmophagus*.

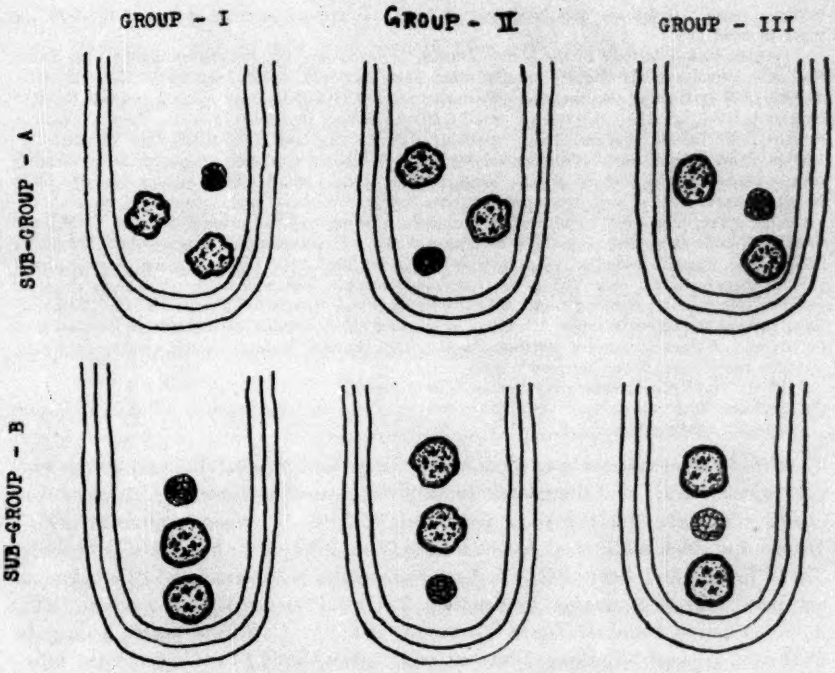



FIGURE 1

Morishita (1924) described 2 species of *Cyclocoelum* viz. *C. distomatum* and *C. vagum*, and placed them in this genus even though they have a rudimentary acetabulum. The presence of an acetabulum is a fairly important character, and warrants the formation of a separate genus for these 2 forms. Provisionally, however, I recommend the retention of these 2 species in the genus *Cyclocoelum* until their life histories are known, which may throw light on their true affinities.

I shall now describe a new species of the genus *Cyclocoelum* which comes in the group with the ovary distinctly pretesticular and the sub-group with the gonads forming 3 points of a triangle.

*Cyclocoelum titiri* n. sp.
(all measurements in millimeters)

Thirty specimens of this parasite were collected from the body cavity of a spur-winged plover, *Haplopterus ventralis* (called titiri in vernacular), caught at the village of Junshi, near Allahabad. Of the 10 birds examined only 1 was found infected with these parasites. Living specimens were white, with greyish brown intestinal contents, and showed slight movement of contraction and expansion in the anterior body.

The tongue-shaped body is 6.075–7.92 long and 1.75–1.905 in miximum breadth, in the gonad region. Cuticle aspinose; oral sucker terminal, transversely oval, 0.165–0.195 long and 0.21–0.27 wide. There is a muscular ridge on the dorsal side of the mouth which Kossack regarded as a generic character. Prepharynx very short, almost absent; pharynx well developed, highly muscular, 0.315–0.33 long and 0.3–0.375 wide; esophagus 0.43–0.45 long visible in some specimens, but almost invisible in most of permanent mounts, in which the bifurcal zone is much displaced anteriorly; intestinal ceca run almost parallel to body wall, posteriorly confluent as an arc 0.195–0.37 in front of the hind end. Excretory bladder large, 0.135–0.255 long and 0.42–0.69 wide

between the intestinal arc and hind end of body. Excretory opening dorsal, 0.135–0.195 in front of hind end.

Gonads in last quarter of the body. Outline of testes smooth, situated obliquely near intestinal arc; anterior testis slightly smaller than posterior, 0.255–0.405 long by 0.255–0.345 wide, at 0.81–1.095 in front of the hind end. Posterior testis 0.58–0.75 in front of hind end and 0.3–0.45 long by 0.315–0.525 wide. Cirrus sac small 0.255–0.375 long by 0.12–0.15 wide. Genital opening median, 0.075 behind pharynx; ovary globular, 0.21–0.3 long and 0.195–0.255 wide situated 1.2–1.65 in front of hind end. Oviduct arising from posteroventral side of ovary, soon forming oötype surrounded by Mehlis' gland. Receptaculum seminis small, 0.06–0.09 long by 0.083–0.135 broad. Laurer's canal well developed. Uterus highly convoluted and occupying almost all the available space, intercecal, cecal, and extra-cecal. Uterine coils in anterior third of body, only intercecal, coils extending posteriorly to intestinal arc. Metraterm well developed, 0.33 long by 0.09 broad. Eggs thin-shelled, ellipsoidal, 0.12–0.15 by 0.045–0.06. Fully matured eggs in distal part of uterus slightly more elongated containing embryos with eye spots. Vitellaria of small follicles, extracecal, extending forward nearly to intestinal bifurcation and posteriorly to hindermost ends of the intestinal arc. Vitellaria in anterior third of body clearly visible but obscured by uterus. Paired transverse vitelline ducts uniting behind posterior testis to form common vitelline duct, latter lying between testes.

*Host: Haplopterus ventralis* (spur-winged plover).
*Habitat:* Body cavity.
*Locality:* Allahabad (U. P. India).

The following monostomes come within the genus *Cyclocoelum* in the group with pretesticular ovary and the gonads forming 3 points of a triangle: 1. *Cyclocoelum similis* (Stossich, 1902) Joyeux and Baer, 1927; 2. *C. tringae* (Stossich, 1902) Joyeux and Baer, 1927; 3. *C. taxorchis* Johnston, 1917; 4. *C. kossacki* (Witenberg, 1926) Joyeux and Baer, 1927; 5. *C. pseudocotyleum* (Witenberg, 1926) Joyeux and Baer, 1927; 6. *C. dollfusi* Tseng, 1930; 7. *Corpopyrum capellae* Yamaguti, 1933; 8. *Corpopyrum longisacculatum* Yamaguti, 1933; 9. *Uvitellina tageri* Yamaguti, 1933; 10. *U. macroisophaga* Hannum and Wilson, 1934; 11. *Cyclocoelum nebularium* Khan, 1935; 12. *C. jaenschi* Johnson and Simpson, 1940.

In light of the discussion given above about the synonymy of various genera of Witenberg with the genus *Cyclocoelum,* in accordance with the views of Joyeux and Baer, and Lal, the following species from the above list are assigned to the genus *Cyclocoelum* and the following new combinations result: *Cyclocoelum capellae* (Yamaguti, 1933); *C. longisacculatum* (Yamaguti, 1933); *C. tageri* (Yamaguti, 1933); *C. macroisophaga* (Hannum and Wilson, 1934).

*C. nebularium* Khan, 1935 is the only species known so far from India which belongs to the group having the ovary pretesticular with 3 gonads forming a triangle. *C. titiri* described here is the second species from India to fall into such a category. The new species can easily be differentiated from *C. nebularium* by the different shape and size of the body, by more anterior separation of the ovary from the testes and the uterus extending further behind, nearly to the posterior end of the body beyond the posterior margin of the gonads (in *C. nebularium* the uterus extends posteriorly almost to the posterior testis). The new species also differs in having the vitellaria joined at the hind end of the body.

*C. titiri* resembles *C. dollfusi* Tseng, 1930, in having general topography, arrangement of the gonads, profuse development of the uterus which extends posteriorly to the hind end of the body and the vitellaria being joined together posteriorly; but *C. titiri* can easily be differentiated by the different shape and size of the gonads (testes in *C. titiri* are smaller, more or less spherical with smooth margins, while in *C. dollfusi* they are larger, broader than long, and lobed); the presence of a well developed oral sucker (poorly developed in *C. dollfusi*); difference in the ratio of

the sizes of the ovary to testes (ovary in *C. titiri* is almost equalling the anterior testis, while in *C. dollfusi* it is much smaller than either testis).

*C. similis* (Stossich, 1902) Joyeux and Baer, 1927, which resembles *C. dollfusi* Tseng, 1930 can also, for the same reasons, be differentiated from the new species.

*Corpopyrum capellae* Yamaguti, 1933, *Corpopyrum longisacculatum* Yamaguti, 1933, *Uvitellina tageri* Yamaguti, 1933 and *U. macroisophaga* Hannum and Wilson, 1934, which are assigned to the genus *Cyclocoelum*, resemble the new species in having the same general topography and belonging to the same group and sub-group. The new species can very easily be differentiated from all the species mentioned above in having the uterus extended laterally in the extra-cecal region and posteriorly to the hind end of the body (uterine coils in all the above species are intercecal and do not extend to the hind end); the vitellaria are joined posteriorly, and the genital opening is slightly distant from the pharynx.

*C. titiri* resembles *C. tringae* Stossich, 1902 in general topography and similar disposition of the gonads but can easily be differentiated by the different shape and size of the body, in having the uterus extending to the extra-cecal fields and to the hind end of the body; the genital opening is slightly separated from the pharynx and the ovary is almost the same size as the anterior testis.

The new species can be differentiated from *C. pseudocotyleum* (Witenberg, 1926) Joyeux and Baer, 1927 by the different shape and size of the body, a Laurer's canal and the uterus extending to the extra-cecal region and to the hind end of the body (uterus in *C. pseudocotyleum* never extends posterior to the testes nor in the extra-cecal fields).

*C. titiri* resembles *C. brasilianum* Stossich, 1902 in general topography; both belonging to the same group and sub-group, they also agree in the union of the vitelline follicles posteriorly, but *C. titiri* can easily be differentiated in having a larger oral sucker and pharynx (poorly developed in *C. brasilianum*); the uterus is extensively developed, extending nearly to the hind end of the body (uterus restricted in front of the gonads in *C. brasilianum*) and in having a well developed Laurer's canal.

ACKNOWLEDGMENT

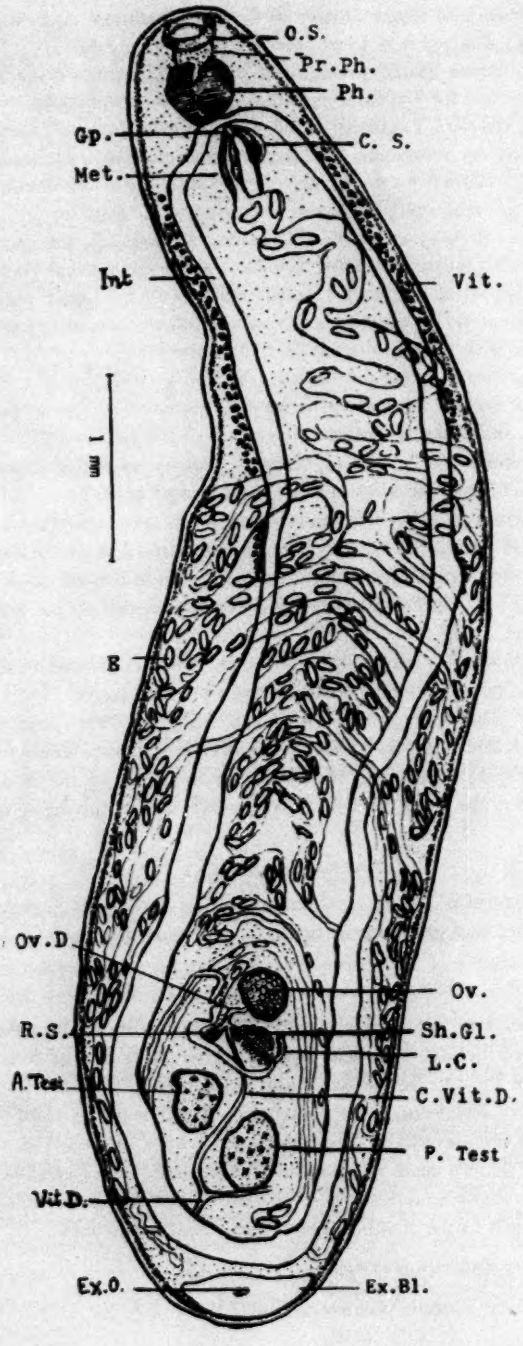
I am very grateful to Dr. B. S. Chauhan, of the Zoological Survey of India, for his valuable advice and guidance in bringing this work to fruition.

SUMMARY

There has been much controversy regarding the validity of different sub-families of the family *Cyclocoelidae* Kossack, 1911. In this paper I have considered *Cyclocoelinae* Stossich, 1902 to be the only valid sub-family.

Similarly there has been much controversy regarding the validity of different genera known in the sub-family *Cyclocoelinae*. I have discussed in this paper the validity of only 2 genera viz. *Cyclocoelum* Brandes, 1892 and *Typhocoelum* Stossich, 1902 and with a slight modification of Morishita's (1924) and Lal's (1939) view I have divided each of the 2 valid genera into 3 distinct groups:

Group I: Ovary distinctly pretesticular.
Group II: Ovary distinctly post-testicular.
Group III: Ovary distinctly between the testes.


O.S.
Pr.Ph.
Ph.
Gp.
C. S.
Met.
Int
Vit.
1 mm
B
Ov.D
Ov.
R.S.
Sh.Gl.
L.C.
A.Test
C.Vit.D.
P. Test
Vit.D.
Ex.O.
Ex.Bl.

Each of the above three groups have been further subdivided into two sub-groups:

Sub-group A: 3 gonads arranged to form three points of a triangle.
Sub-group B: 3 gonads are arranged in a straight line.

Further, I have described in this paper a new species of the genus *Cyclocoelum* which comes under group I where ovary is pretesticular and sub-group A where the 3 gonads form 3 points of a triangle.

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### Explanation of Plate

A.Test., anterior testis; C.S., cirrus sac; C.vit.D., common vitelline duct; E., egg; Ex.Bl., excretory bladder; Ex.O., excretory opening; Gp., genital pore; Int., intestinal ceca; L.C., Laurer's canal; Met., metraterm; O.S., oral sucker; Ov., ovary; Ov.D., oviduct; P.Test., posterior testis; Px., pharynx; Pr.Ph., prepharynx; R.S., receptaculum seminis; Sh.Gl., Mehlis' gland; Vit., vitellaria; Vit.D., vitelline duct.

## RESEARCH NOTE

### AN APPARATUS FOR ARTIFICIAL FEEDING OF SIPHONAPTERA

In connection with an investigation into the host specificity of Siphonaptera, an apparatus was designed for the artificial feeding of these insects through a membrane. This apparatus appeared to operate successfully in preliminary tests and will be of interest to persons concerned with the rearing of fleas in the laboratory.

The feeding apparatus is illustrated in the accompanying diagram. In operation water is used to warm the blood which the plastic dish contains. Beef blood was maintained slightly below normal human body temperature in our experiments.

Fleas are placed in the feeding tube and this is inserted through the wooden cover, so that the membrane is in contact with the blood. A rubber washer near the top of the tube rests on the wooden cover, holding the tube in place. A hole in the cap of the feeding tube allows for ventilation. Escape of the insects is prevented by a piece of fine muslin, taped to the underside of the cap.

Three membranes have been used successfully in our investigation. Silver light and gold beater membranes are artificial. Natural pig bladder was also used.

This apparatus can be constructed inexpensively. Any water bath may be substituted for the Technicon constant temperature bath. The plastic dish can be bought in any store which sells household goods. The feeding tubes were made by cutting the bottoms from plastic vials, purchased at a drug store.

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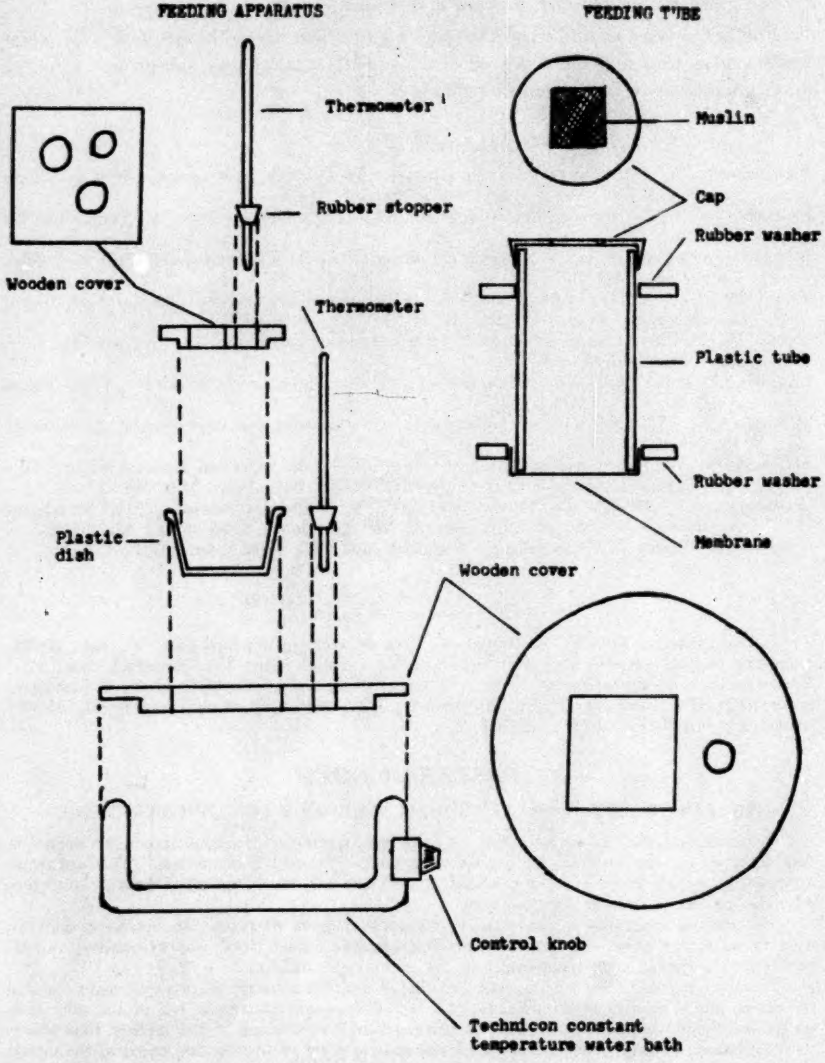
Benton, New York State College for Teachers, Albany.—ROBERT H. CERWONKA, *Greenville Central School, Greenville, N. Y.*, AND RAYMOND A. CASTILLO, *University of Maine.*




## RESEARCH NOTE

### THE STATUS OF THE TREMATODE GENUS *BIANIUM* STUNKARD, 1930, A SYNONYM OF *DIPLOPROCTODAEUM* LA RUE, 1926.

One of us, (F.S.), collected specimens of a trematode from *Ceratacanthus scripta* (= *Osbeckia scripta*) in Bimini, B.W.I. The trematodes were at first believed to be specimens of *Bianium lecanocephalum* Perez-Vigueras, 1955, described from *Osbeckia scripta* in Cuba. In view of the striking similarity of our trematode specimens with *Diploproctodaeum haustrum*

(McCallum, 1918) LaRue, 1926, McCallum's type material (U.S. Nat. Mus. Helm. Coll. No. 36301) was recalled for study. It became immediately apparent that *D. haustrum* (McCallum, 1918) La Rue, 1926, was identical with *Bianium lecanocephalum* Perez-Vigueras, 1955. *D. haustrum* (McCallum, 1918,) La Rue, 1926, was originally collected from *Alutera schoepfi* (= *Ceratacanthus schoepfi*) in the southeastern United States. Reexamination of specimens of *Bianium plicitum* (Linton, 1928) Stunkard, 1931, from puffers collected by one of us, (F.S.), in the American Atlantic and Tropical American Pacific were compared with *D. haustrum* (McCallum, 1918) La Rue, 1926. There is not a single character upon which 2 seperate genera can be established for these 2 species. *Bianium* Stunkard, 1930 is clearly a synonym of *Diploproctodaeum* La Rue, 1926.

The genus *Diploproctodaeum* La Rue, 1926, has been the source of much taxonomic and nomenclatorial confusion for the last 30 years. McCallum (1918, Zoopath. **1**: 77–98) named *Hemistomum haustrum* from *Ceratacanthus schoepfi*. La Rue (1926, J. Parasitol. **12**: 207–209) recognized that *Hemistomum haustrum* McCallum, 1918, had been improperly assigned to the genus *Hemistomum* and named the genus *Diploproctodaeum* for this species of McCallum. *H. haustrum* McCallum, 1918 became *Diploproctodaeum haustrum* (McCallum, 1918) La Rue, 1926. Linton (1928, Proc. U.S. Nat. Mus. (2722) **73**: 1–36) named a new trematode, *Psilostomum plicitum*, from a gull in Woods Hole, Mass. The occurence of *P. plicitum* Linton, 1928, in a gull was apparently an accidental infection because the species was later found to live almost exclusively in puffers. *Diploporus* Ozaki, 1928, was named for 2 species of trematodes living in puffers from Japan. *Diploporus* Ozaki, 1928, is preoccupied by *Diploporus* Von Buch, 1845, and *Diploporus* (Troschel, MS) Martens, 1866. The species described in *Diploporus* Ozaki, 1928, are congeneric with *Diploproctodaeum haustrum* (McCallum, 1918) La Rue, 1926, and *Psilostomum plicitum* Linton, 1928. The genus *Bianium* Stunkard, 1930, was named with *Bianium concavum* Stunkard, 1930, as type species. *Bianium concavum* Stunkard, 1930, was later found to be identical with *Psilostomum plicitum* Linton, 1928. Stunkard (1931; Zeitschr. Parasitenk. **3**: 713–725) corrected this error in identification and *Psilostomum plicitum* Linton, 1928, became *Bianium plicitum* (Linton, 1928) Stunkard, 1931, type species, (Syn.: *Bianium concavum* Stunkard, 1930, by reason of date priority). Linton (1928), Ozaki (1928, Jap. J. Zool. **2**: 5–53), and Stunkard (1930, Anat. Rec. **47**: 363; 1931) were obviously unaware of La Rue's (1926) description of *Diploproctodaeum*.

The genus *Diploproctodaeum* La Rue, 1926 contains the following species: *Diploproctodaeum haustrum* (McCallum, 1918) La Rue, 1926, (Syns.: *Hemistomum haustrum* McCallum, 1918; *Bianium lecanocephalum* Perez-Vigueras, 1955, new synonymy); *Diploproctodaeum plicitum* (Linton, 1928) n. comb., (Syns.: *Psilostomum plicitum* Linton, 1928; *Bianium concavum* Stunkard, 1930; *B. plicitum* (Linton, 1928) Stunkard, 1931; *B. adplicatum* Manter, 1940); *Diploproctodaeum cryptostoma* (Ozaki, 1928) n. comb., (Syns.: *Diploporus cryptostoma* Ozaki, 1928; *Bianium cryptostoma* (Ozaki, 1928) Manter, 1947); *Diploproctodaeum hemistoma* (Ozaki, 1928) n. comb. (Syns.: *Diploporus hemistoma* Ozaki, 1928; *Bianium hemistoma* (Ozaki, 1928) Yamaguti, 1934); *Diploproctodaeum holocentri* (Yamaguti, 1942) n. comb., (Syns.: *Bianium holocentri* Yamaguti, 1942; *B. hemistoma* (Ozaki, 1928) Yamaguti, 1934 of Caballero, Bravo, & Grocott, 1952, and of Perez-Vigueras, 1955); *Diploproctodaeum tetraodontis* (Nagaty, 1956) n. comb., (Syn.: *Bianium tetraodontis* Nagaty, 1956).

*Diploproctodaeum haustrum* (McCallum, 1918) La Rue, 1926, differs from *D. plicitum* (Linton, 1928), by possessing a more sagittate forebody and a consistently longer esophagus. It may be that upon a study of larger series of *D. haustrum* (McCallum, 1918) La Rue, 1926, the species will be found to be identical.

The genus *Diplocreadium* Park, 1939, has been considered a synonym of *Bianium* Stunkard, 1930, by Yamaguti (1953, Systema Helminthum, Tokyo, Japan). Yamaguti believes that the ani of *Diplocreadium* were probably overlooked. There are other genera of lepocreadiids with a scooped-out forebody, but without ani (viz. *Choanodera* Manter, 1940). We prefer to retain the genus *Diplocreadium* Park, 1939, until such time as ani are demonstrated.

Acknowledgments are here extended to the following: For support received by one of us, (F.S.), to the Lerner Marine Laboratory of the American Museum of Natural History, where our specimens of *Diploproctodaeum haustrum* (McCallum, 1918) La Rue, 1926, were collected; to Dr. G. R. La Rue for advice and information concerning the whereabouts of McCallum's specimens of *D. haustrum* (McCallum, 1918) La Rue, 1926; and to Mr. Allen McIntosh for the loan of U. S. Nat. Mus. Helm. Coll. Specimens No. 36301.—FRANKLIN SOGANDARES-BERNAL AND ROBERT F. HUTTON, *Florida State Board of Conservation Marine Laboratory,* Maritime Base, Bayboro Harbor, St. Petersburg, Florida.* (* Contribution No. 10)

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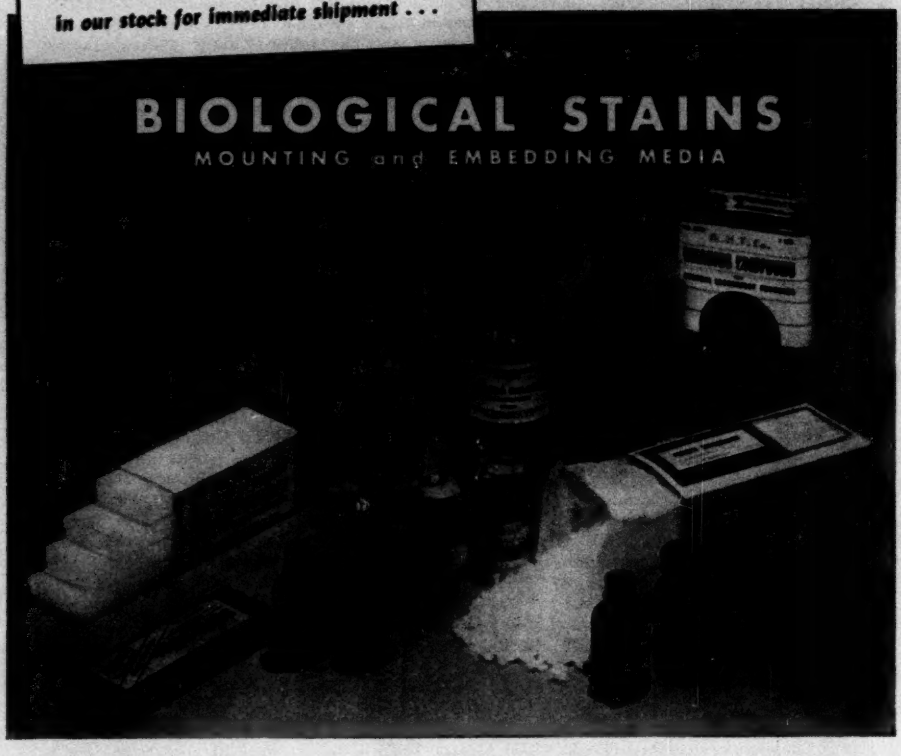


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